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An International Journal of Genetics

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An International Journal of Genetics

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PATTERNS OF CHROMOSOME BREAKAGE AFTER IRRADIATION AND AGEING

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1. INTRODUCTION

SINCE the discovery of the mutagenic effects of mustard gas in 1942, numerous groups of chemical substances have been shown to be active in breaking chromosomes (Loveless and Revell, 1949). In general the pattern of breakage is very similar to that induced by high energy radiations, although some authors (*e.g.* Ford, 1949; Revell, 1953; McLeish, 1953) claim to have discovered certain patterns of localisation of breaks after chemical treatments which are not apparent in X-irradiated material. Notwithstanding these reported differences in breakage pattern, the close similarity of chemical and X-ray induced breakage is hardly to be expected on a target theory as developed by Lea (1946). However, similarity would be expected if X-rays work through a chemical phase by the production of mutagenic substances or radicals which have a definite and perhaps prolonged half-life period before destruction in the cell (Gray, 1951).

The study of X-ray induced chemical reactions in watery media such as cytoplasm is still in its infancy (Read, 1951). That the chemical phase is of critical importance in understanding irradiation damage to chromosomes is now shown by numerous lines of evidence. They include :

(1) the similarity in pattern of spontaneous and X-ray induced breakage and mutation ;

(2) the complex interactions of treatments combining a pre-treatment with infra-red, ultraviolet or chemical mutagen with X-rays ;

(3) the dosage time and fractionation experiments which were initially interpreted as being due to rapid re-union and " healing " after hits on the chromosome target ;

(4) the importance of oxygen and temperature in controlling the efficiency with which X-rays break chromosomes ;

(5) the demonstration that prior irradiation of the medium on which bacteria, *Neurospora* spores, or *Paramecium* are subsequently plated out, increases mutation rate ;

(6) the existence of genetically different strains of differing X-ray sensitivity, in bacteria, viruses and *Drosophila* ;

(7) the fact that strains which have been selected for resistance to X-rays often show resistance to chemical mutagens and vice versa.

If the radicals and mutagenic substances produced by ionisation after X-irradiation have an extremely short life, the target theory will hold as a limiting case. Evidence is accumulating that these substances may have a long life, and we must expect the target theory not to hold generally.

It is surprising that although claims are made that X-irradiation and chemical breakage show the same (or different) patterns little attempt has been made since the early days of experimentation with *Drosophila*, to determine the exact and detailed quantitative distribution of breaks after various treatments. In this paper we shall discuss the following problems :

(*a*) the distribution of breaks along a chromosome ;

(*b*) the distribution of breaks between chromosomes of one nucleus ;

(*c*) the distribution of breaks between cells.

The target theory in its crudest form should predict a random distribution both between and within chromosomes. Chemical breakage, being presumably a less energetic process, might disclose weak chromosomes or weak regions within a chromosome.

Most of the previous analyses of break distribution have started with the assumption, that breaks both within and between arms are distributed at random, *e.g.* Newcombe (1942), Catcheside, Lea and Thoday (1946). Any non-randomness is then explained by a non-random re-union caused by polarisation and the movements of the broken chromosomes, or by the complication introduced into the measurement of chromosome lengths in *Drosophila* by heterochromatin. Many of these data might easily be explicable by assuming a non-random distribution of breaks between chromosomes and chromosome arms.

On the other hand, Sax and Mather (1939) and Swanson (1942) claim to have found a non-random distribution of breaks along *Tradescantia* chromosomes ; and Koller and Ahmed (1942) have demonstrated that both induced and spontaneous breakages in the third chromosome of *Drosophila* are non-randomly distributed. Camara (1941) has given a thorough analysis of the X-ray breakage pattern in *Triticum monococcum* and shows that chromosomes are more easily broken by X-rays at the ends and near the centromere.

The distribution of breaks between cells after X-irradiation is expected to be Poissonian on the basis of a direct action theory, but need not be so after a chemical treatment which might involve thresholds and unequal dosages in addition to cumulative action.

Lea (1946) gives three sets of data which show a Poissonian distribution of breaks between cells. The mean number of breaks per cell is not given by Lea but according to his table 56, the mean varies between 0·25-0·55. Lea has not attempted to analyse Newcombe's (1942) more extensive data from this simple standpoint. As will be shown later, his data give an approximately Poissonian

distribution with a mean of one break per cell ; but as the mean break frequency is increased by increasing X-ray dosage, the distribution departs significantly from a Poissonian distribution, overdispersion becoming gradually more pronounced. These deviations are in the same direction as those occurring in X-rayed *Drosophila* sperm as analysed by Lea (loc. cit., p. 219). Darlington and La Cour's (1945) data on X-rayed *Trillium* roots show the same tendency to overdispersion. For chemical breakage few data are available. Darlington and Koller's (1947) distributions after mustard gas treatment show overdispersion. For spontaneous breakage in *Trillium* (Sparrow, 1949) and *Tulipa* (Darlington and Upcott, 1941) overdispersion is very pronounced.

Allium seed is suitable material for use in experiments on ageing and so can form a useful basis for the study of spontaneous breakage. Cytologically, it offers certain general difficulties to a complete analysis of breakage because of the number, lengths and similarity of the chromosome arms. It has the advantage of large chromosomes not possessing any visible heterochromatin regions which may complicate breakage patterns. Ford (1949), Revell (1953), McLeish (1953) and others have shown localisation of breaks in the heterochromatin after chemical treatments.

Because most divisions are in side view in root-tip preparations, scoring is more difficult than in pollen grain mitosis where ease of preparation and the haploid set are additional advantages. Notwithstanding these limitations, the root tip material from treated seeds has some outstanding advantages over the more commonly used pollen grain division.

Recent work including in particular that of Sparrow (1951), Lane (1953) and Haque (1953), has shown that the physiological conditions of the cell interact in many ways with the treatment producing breakage. The observable effects show that the cell has phases of quite varied " sensitivity " dependent on the type of treatment and the condition of the nucleus at the time of treatment. The treatment affects cell physiology, which in turn affects the observed result. Both sister re-union and non-sister re-union values are changed and these in turn affect the scored result. Thus, many experiments fail to give concise and consistent results because of unidentified biological interactions. In view of our inadequate knowledge of these interactions, any experimental methods which avoid or restrict these effects is preferable. If the purpose of the analysis is the study of primary breakage, then treatment of the fully dormant seed allows a directness which is not possible using other material. Dormant embryos afford stable and comparatively uniform conditions during treatment. An almost indefinite controlled delay can be made between treatments and fixation, thereby lessening physiological interactions. Lastly, the cells can be given a wider range of treatment with less physiological damage. The low non-sister re-union values and the

virtual absence of chromatid breakage, quite apart from other advantages, make the material ideal in the study of primary breakage.

2. MATERIALS AND METHODS

Dormant seed of *Allium cepa* var. Brown Spanish was used throughout the experiment. Three stocks were used. The first was from the 1950-51 season for the irradiation and control experiments. This seed was not more than six months old at the time of experiments. The others were two and four years old respectively. The two-year stock had such a low frequency of breakage that a detailed analysis was not carried out. Six months before observations started each of the aged stocks was divided into two lots, one of which was stored at room temperature and the other at 3° C. This difference in storage had a marked effect on the four-year-old seed but no appreciable effect on the two-year-old. The period of storage at low temperature so markedly increased chromosome breakage in the four-year-old material that scoring was almost impossible. All subsequent data on aged seed thus refer to the four-year-old seed stored at room temperature since collection.

X-rays were generated by a Coolidge type tube operating at 22·4 kV (Peak) at 55 mA, giving an intensity of 50r sec^{-1} at 10 cm. from the target. The dose was measured by a thimble type Victoreen meter at the same distance from the target, the dose being regulated by varying the duration of irradiation. Doses of 200, 400, 800, 1200, 1600 and 2400r were given, those above 400r being administered in fractions of eight seconds, with ten seconds between fractions. The temperature at the time of irradiation was 18° C.

The highest dose (2400r) was the only dose analysed completely as it gave a mean breakage level per cell approaching that in aged seed.

Treated seeds and controls were germinated on moist filter paper at 23° C. Radicals in the length range 3-5 mm. on the fifth day of germination were selected, fixed in acetic alcohol and stained in Feulgen. Within any one treatment all tips were handled in the same tube to avoid any differences in staining technique.

Sampling. Fifty root tips of each of the treatments, fresh seed, irradiated fresh seed, and aged seed were prepared as squashes. Sampling was conducted at random from the fifty slides within each treatment.

No breakage was observed in any of the fresh material. The aged material showed a marked heterogeneity in the degree of breakage between tips, as compared with the irradiated material. In both these treatments slides withdrawn were completely scored for divisions and breakage. Sampling was continued until the total number of acentric fragments in each treatment approximated 250. It was coincidental that seven slides were used in each case. Preliminary examination had shown the average level of breakage to be approximately the same. Quite apart from the enormous increase in labour involved, it was thought unwise to select for uniformity within the aged material, because the artificial removal of the heterogeneity would possibly limit the usefulness of the comparison between the two treatments. This heterogeneity is expected to make the analysis of the number of acentrics per cell (N) difficult, since the data from various roots must be treated separately. The heterogeneity is not expected to have any effect on the distribution of acentric length (X) within classes of (N). The essential feature of the sampling method was to maintain comparable totals for both number of cells showing breakage and number of acentrics in both treatments.

Scoring. Roots were selected so that fixation coincided with the maximum initial division rate in the meristem, thus ensuring that none of the cells had passed through two divisions since treatment. Squashes were examined for :

(1) number of divisions in anaphase or early telophase ;
(2) number of divisions in anaphase or early telophase showing chromosome breakage ;

(3) number of acentrics (N) ;
(4) length of acentrics (X) ;
(5) SR in acentrics ;
(6) SR and NSR in centrics.*

As mentioned in the introduction, there is very little chromatid breakage. Re-union, especially NSR, is very low (see table 1 showing the classified aberration

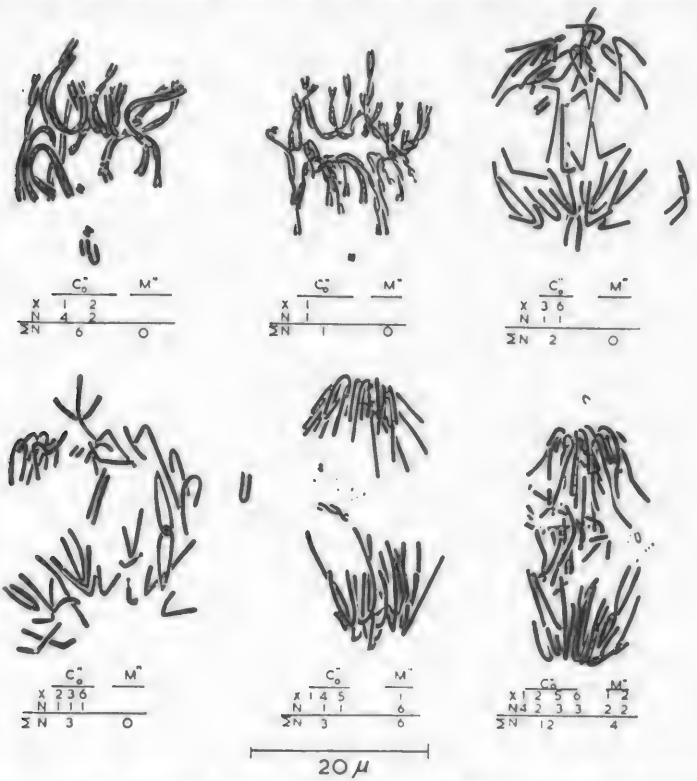

FIG. 1.—Mitotic divisions in root tip cells of germinated aged seed of *Allium cepa* scored for number, N, and length, X, of acentric fragments C_0'' and minutes m″.

types). In the centrics SR is rarely separable from NSR, because of the uniformity in the chromosome morphology. Interchanges involving the trabant chromosome as illustrated in fig. 1, represent the only cases which may be scored with accuracy. The only practical measure of re-union for centrics is the frequency of polycentrics and since this includes both SR and NSR, it is of little use in analysis.

* We shall use Darlington and Upcott's (1941) abbreviations and terminology. They are :

C_0—acentric ; C_1 etc.—monocentric, dicentric, etc.
C′—chromatid fragment ; C″—chromosome fragment.
1 SR, 2 SR—single or double sister re-union respectively.
1 NSR, 2NSR—single or double non-sister re-union respectively.
r—ring ; m″—paired minute.

Both number and length of acentrics can be scored with a high degree of accuracy and afford the simplest and most direct measure of the initial breakage.

Apart from the occasional failure to detect very small fragments (minutes and subminutes), there are two possible cases where errors in determining N may arise. These are in the cells on the extremes of breakage effects. There is a greater chance of non-detection where breaks are few, since divisions without obvious fragments tend to be passed over without the rigorous examination given to cells showing breakage. Also there is a known number of cells where the number of breaks

TABLE 1

Types of aberrations

	Tip	Divisions scored			Acentrics						Centrics			
		Total	Broken	F% *	C_0'	C_0''	C_0'' 1 SR	C_0'' 2 SR	$C_0''r$ 1 R	m'' 1 R	C_1'' 1 SR	C_2'' 1 R	$C_1''2r$ 1 R	$C_1''r$ 1 R†
Aged seed	1	206	4	2	...	7	1	...	...	...	...	...	...	...
	2	177	14	15	...	18	4	1	...	13	4	...	...	...
	3	270	2	1	...	5	...	...	...	...	...	...	...	...
	4	105	20	21	1	46	3	...	1	8	7	...	...	...
	5	200	7	3	...	10	...	...	...	9	2	...	...	...
	6	76	23	76	1	73	7	1	...	5	13	1	1	...
	7	174	33	14	...	64	2	1	...	3	6	...	...	...
Total	7	1208	103	9	2	223	17	3	1	38	32	1	1	...
							243							
Fresh seed X-rayed (2400r)	1	207	22	11	1	31	3	1	1	1	3	1	...	...
	2	252	16	6	...	22	4	1	...	...	7	...	1	...
	3	322	49	15	2	46	5	2	...	10	8	...	...	...
	4	217	27	12	...	30	4	...	...	4	1	...	...	...
	5	195	21	11	...	27	1	...	...	5	1	...	...	...
	6	236	16	7	...	20	3	...	...	6	5	...	...	...
	7	105	22	21	...	28	3	1	...	3	3	...	...	1
Total	7	1534	173	11	3	204	23	5	1	29	28	1	1	1
							232							

* This column gives the frequency per cent. of broken cells including those unscorable, as a measure of net breakage for estimation of heterogeneity.

† SR = sister re-union. R = non-sister re-union. Where type of union is ambiguous the more probable condition is assumed.

exceeds 15 to 20, the disorganised mass of fragments being beyond scoring with accuracy. In only a few tips of the aged material do the numbers of such cells exceed 5 per cent. of the scorable divisions.

Natural variation in chromatid contraction renders extreme accuracy unnecessary in the measurement of acentric length. Where fragments were oblique to the optical axis, true lengths were calculated from the apparent lengths measured on the ocular micrometer and the vertical height—given by readings of the fine adjustment spindle micrometer under restricted depth of field conditions. Fragment lengths were grouped to the nearest μ, which is well within the range of contraction. It is not possible to relate fragments to specific chromosomes, or parts of chromosomes, as Camara has done in *Triticum*, or as McLeish and Revell have done in *Vicia*. Nor is it possible to obtain specific cell constants for the degree of contraction,

owing to the difficulties of identifying particular arms consistently. These limitations can, however, be largely overcome by a simple statistical treatment of the data, which will be discussed with the results.

Minutes (m'') are separated from fragments (C_0'') on diameter. All fragments have been scored as C_0'' if the diameter is the same as the chromatid diameter. Most of the minutes are identical paired bodies and show no sister re-union. They are assumed to be paired rings. The diameter of the thread is always less than half the chromatid diameter.

3. TYPES OF BREAKAGE

The classified results of scoring are shown in table 1. The similarity between the aged seed and the irradiated fresh seed is at once apparent. The outstanding difference is the heterogeneity in the breakage level between roots in the aged seed. Germination in the aged seed is, of course, reduced compared with that of fresh seed. Post-germination death is also evident in the aged seed. The irradiation of fresh seed by doses of the order of 3 Kr. reduces its viability by only a small amount.

The pattern of aberration types is strikingly similar. Chromatid breakage is doubtfully present; SR is low and NSR almost absent; micro-acentrics have a fairly high frequency of occurrence. Fig. 1 shows some typical divisions from aged seed material and illustrates the scoring conventions used.

This general pattern is remarkably different from that obtained in the irradiation of actively dividing root tips, where chromatid breaks and re-unions of various types are frequent. More surprisingly, it shows marked differences from the results obtained by irradiating pollen grains in the immediate post-meiotic stage. Although the nuclei are in an apparent resting condition in pollen, NSR is frequent and the proportion of simple deletions is lower than in the dormant seed material.

Tables 2-8 give the data in a form suitable for consideration of the four problems of the distribution of breakage within chromosomes, between chromosomes of the same nucleus, between nuclei and between root tips.

4. DISTRIBUTION OF BREAKS WITHIN AND BETWEEN CHROMOSOMES

Table 2 and fig. 2 give the distribution of length in acentric fragments. The distributions in aged and X-rayed cells are very similar in form, most fragments (*c.* 60 per cent.) being less than 25 per cent. of the length of the average chromosome arm. There is a subsidiary maximum frequency at about the mean arm length (6·5 μ).

The detailed analysis of these distributions requires the calculation of the distribution expected assuming a random position of breakage along the length of all chromosomes. For any particular chromosome

arm ν, and for only one break, the expected distribution of acentric length (X) is obviously a simple rectangular distribution,

$$dp = \frac{1}{l_\nu}\,dx$$

where $l\nu$ is length of chromosome arm.

TABLE 2

Frequency distribution of X, length of acentrics C_0''

Total distributions given for (*a*) (N > 1) one or more fragments per cell.
(*b*) (N = 1) one fragment per cell.

	Root tip	Length of acentrics C_0'' grouped to the nearest integral μ												f_x	$\bar{X}_x$	V_x
		1	2	3	4	5	6	7	8	9	10	11	12			
Aged seed .	1	4	1	1	...	1	...	...	...	1	...	...	...	*8*	2·9	8·1
	2	9	4	2	3	3	2	...	...	...	...	...	...	*23*	2·7	3·2
	3	2	...	2	...	...	...	1	...	...	...	...	...	*5*	2·8	6·5
	4	26	6	7	...	5	5	...	...	1	...	...	...	*50*	2·5	4·3
	5	1	3	3	2	1	...	...	...	...	...	...	...	*10*	2·9	2·1
	6	24	18	20	2	4	7	2	1	1	...	...	2	*81*	3·0	5·6
	7	39	10	4	2	1	7	2	1	...	...	...	...	*66*	2·3	5·0
Total (*a*) .	N > 1	**105**	**42**	**39**	**9**	**15**	**21**	**5**	**2**	**3**	...	...	**2**	**243**	**2·7**	**4·6**
Total (*b*) .	N = 1	25	8	8	1	2	7	3	1	...	...	...	...	*55*	2·7	4·7
Expected .	N = 1	8·3	8·3	8·3	8·0	7·5	6·2	3·6	3·4	1·0	0·5	...	...	*55*	3·9	6·3
Fresh seed irradiated (2400r)	1	10	8	2	4	2	3	4	2	...	...	...	...	*35*	3·4	5·9
	2	9	11	2	3	...	...	2	...	...	...	...	...	*27*	2·3	2·8
	3	25	10	5	3	4	2	3	1	...	...	...	...	*53*	2·6	5·2
	4	15	5	4	2	...	2	3	2	...	...	1	...	*34*	3·1	7·4
	5	10	8	2	1	2	4	...	...	...	1	...	...	*28*	2·9	5·3
	6	7	4	3	1	1	3	2	2	...	...	...	...	*23*	3·5	6·6
	7	10	7	5	2	...	2	3	1	1	...	...	1	*32*	3·4	7·9
Total (*a*) .	N > 1	**86**	**53**	**23**	**16**	**9**	**16**	**17**	**8**	**1**	**1**	**1**	**1**	**232**	**2·9**	**5·5**
Total (*b*) .	N = 1	60	25	11	8	5	5	7	3	1	1	1	1	*128*	2·7	5·8
Expected .	N = 1	19·2	19·2	19·2	18·6	17·4	14·4	8·4	7·8	2·4	1·2	...	...	*128*	4·0	5·1

Where there are j arms of lengths l_1, l_2, ..., the probability of obtaining a single fragment of length x units from any one of the i arms is the reverse sum polygon of the lengths of the j arms.

$$dp = \sum_{j}^{i} \nu \max\,(l_\nu\,;\,l_j) \Big/ \sum_{1}^{j} \nu \qquad (1 \leqslant \nu \leqslant j)$$

In *Allium* where it is impossible to recognise the donor chromosome arm, this distribution gives the only practical method of determining the distribution expected from random breakage. Table 3 gives the lengths of the 16 chromosome arms of the haploid complement. For

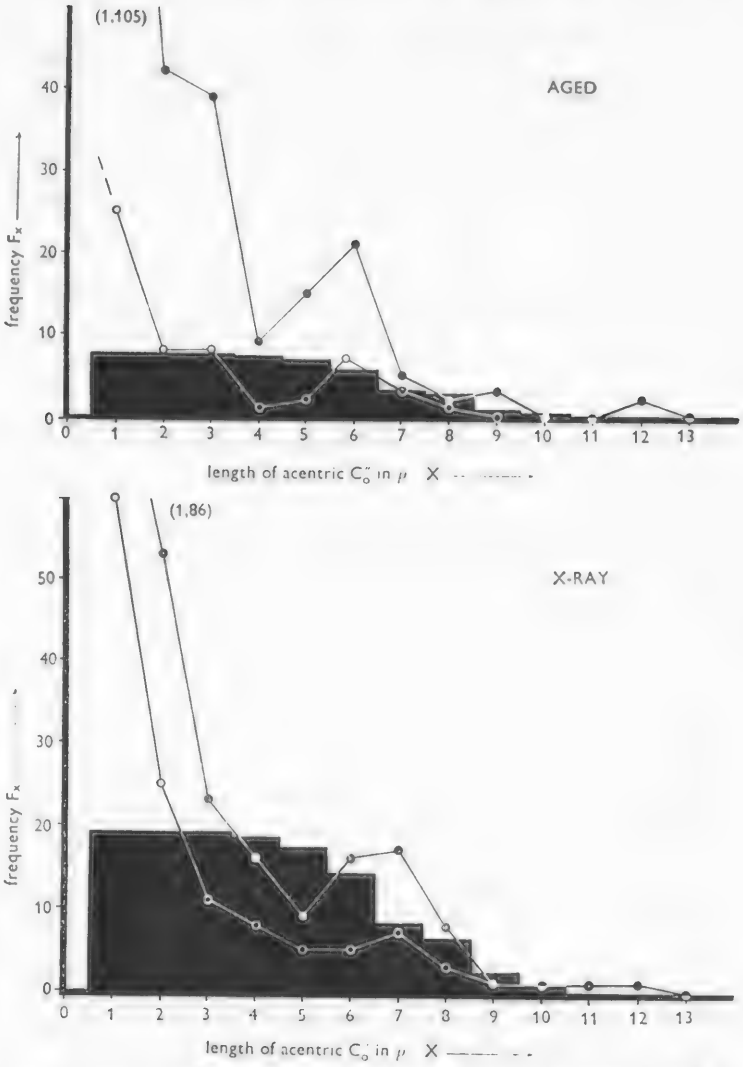

FIG. 2.—Graphs giving data from table 2, showing the frequency distribution length, X, of acentric fragments in cells where (the number of fragments) N⩾1 (blacked circles) and N = 1 (hollow circles). The blacked histogram gives the distribution for N = 1 of fragment length expected if the breaks are distributed at random along the chromosomes.

case of measurement the relative lengths of the arms were determined in slight C-mitosis where the chromosomes are straight and the centromeres easily visible. These relative values were then transformed into actual mean lengths by applying a conversion factor determined by measuring the length of the trabant chromosome in all favourable

TABLE 3

Mean arm length $\bar{l}$ at anaphase in microns

Chromosome	A	B	C	D	E	F	G	H
Arm *a* . .	8·0	9·6	8·0	8·3	7·4	8·4	6·2	6·2
Arm *b* . .	7·6	6·0	6·4	5·2	5·6	3·5	5·1	4·5
Total length .	15·6	15·6	14·4	13·5	13·1	12·4	11·3	10·7

divisions during scoring in both aged and X-rayed root tips. Forty measurements of the trabant chromosome gave a mean length of $12{\cdot}4 \pm 0{\cdot}5\ \mu$.

Table 4 gives the derivation of the probability distribution of length of acentric fragments where only one fragment is present in the cell (N = 1). These distributions are shown as the blacked-in

TABLE 4

Derivation of the probability distribution of acentric fragment length X

Length classes in μ	1	2	3	4	5	6	7	8	9	10
Frequency of l_v	0	0	1	2	5	10	1	9	2	2
Frequency of l_v for $l_v \geqslant X$	32	32	32	31	29	24	14	13	4	2
P (X; where N = 1)	*0·150*	*0·150*	*0·150*	*0·146*	*0·136*	*0·113*	*0·066*	*0·061*	*0·019*	*0·009*

l_v = mean length of the vth arm.
X = length of acentric fragment C_0''.
N = number of fragments C_0'' per cell.

histogram of fig. 2. It is clear from these figures that fragments of length less than 2 μ are in great excess whilst there is a deficiency of fragments of lengths 3-5 μ. Table 5 gives an analysis of these deviations by χ^2 and leaves no doubt as to their statistical significance.

There are four acentric fragments in the two treatments which exceed the mean length of the longest arm. It is possible that these long fragments arise by NSR between shorter fragments. However, variations in degree of contraction of the trabant chromosome give an estimated standard error for long fragments of almost 0·5 μ.

Thus it is likely that these extreme lengths are simply due to normal variations in degree of contraction of the chromosomes. There is, thus, no conclusive evidence of NSR in the acentrics.

Where there is more than one fragment in the cell a donor may contribute more than one fragment. The expected distribution of fragment length for such conditions is complex since for N fragments per cell ($N > 1$), the probability of any arm ν donating any number r, ($1 < r \leqslant N$), of the N fragments is,

$$\left[\frac{l_\nu}{\Sigma l_\nu}\right]^r \cdot \left[\frac{\Sigma l_\nu - l_\nu}{\Sigma l_\nu}\right]^{N-r} = \frac{l_\nu^r (\Sigma l_\nu - l_\nu)^{N-r}}{[\Sigma l_\nu]^N}$$

Since l_ν is small with respect to Σl_ν and the mean N is small with respect to the number of donors, this probability is small if breakage

TABLE 5

χ^2 for grouped classes of acentric fragment length X

Class	1-2 μ	3-5 μ	6-7 μ	8-10 μ	Total
χ^2 Aged N = 1	33·6	10·2	0·2	3·2	47·2
Aged N ⩾ 1	75·0	21·0	4·8	8·8	108·6
χ^2 Irradiated N = 1	88·2	18·5	6·4	3·7	116·7
Irradiated N ⩾ 1	69·0	27·6	3·2	5·2	105·0

per unit length is equal in all chromosomes. However, if there are marked differences in susceptibility between arms, those arms which are more easily broken will have reasonably large values of the above probability. The net effect of such conditions would lead to a marked alteration in the form of the distribution, since if any arm donates r fragments, the length distribution becomes a $\beta(1, r)$ distribution, which for $r > 1$, gives a rapid increase in the probability of short fragments as r increases.

Table 2 and fig. 2 show that the distributions of X are the same for cases of $N = 1$ and $N \geqslant 1$. There is no suggestion that short fragments tend to occur more frequently in cells with a high N. This is also shown by the fact that there is no significant negative correlation between X and N ($r = +0{\cdot}03$, $P = 0{\cdot}7$ for aged seed and $r = +0{\cdot}15$, $P = 0{\cdot}2$ for X-rayed seed). Within the limits of these comparisons it seems safe to conclude that the chance of breakage per unit length does not vary greatly between different chromosomes of the complement. Even if a more precise analysis, *e.g.* by counting breaks in the easily identified chromosomes, as has been done by McLeish (1953) for *Vicia*, should show a range in susceptibility to breakage between chromosomes, this range must be small and substantially the same for aged and X-rayed seed.

It has already been shown that the frequency of NSR in both centrics and acentrics is very low in this material. This means that almost all acentrics are terminal deletions and that a particular length of fragment can be related to a fixed region in the mean arm length. Thus, we may conclude that the chance of breakage (or the chance of persistence of a break until the time of observation) after both ageing and X-ray treatment, is most frequent at the ends of all chromosomes, while mid-arm regions have the least chance of breakage, there being a smaller increase in the chance of breakage in the centromere regions. These findings are very similar to those of Camara (1941) for the trabant chromosomes of *Triticum monococcum*. In *Triticum* the chances of breakage are almost equal near the ends and near the centromere. In *Allium* the chance appears to be much higher near the ends than near the centromere. However, some of this difference may be due to the three-fold ($3{\cdot}5\ \mu$-$9{\cdot}6\ \mu$) range in length of chromosome arms in *Allium*.

Ford (1949, see also Gray, 1951, p. 264) has shown non-random breakage in *Vicia* using nitrogen mustard, but claims, in contrast to the present results, that the distribution after X-irradiation is random. His results seem hardly extensive enough to prove the latter point conclusively.

Similar non-random distributions in *Vicia* have been demonstrated by Deufel (1951), Revell (1953,) and McLeish (1953) using various chemical agents. Revell (1953) states that the X-ray induced breaks in the long chromosome are apparently at random. However, calculations based on data from his diagram do not support this statement. Deufel (1951) obtained a markedly non-random distribution for breaks in the same chromosome using X-irradiation. It is difficult to say without full data, whether there is heterogeneity between the different chemical treatments, or even between these and the X-ray data. In this respect it is interesting to notice that an analysis of breakage in the X-chromosome of *Drosophila* conducted by Fahmy and Bird (1953) indicated that the patterns produced by a diepoxy-butane, an imino-substituted triazine and a sulphur mustard, were not significantly different.

It has been claimed that the localisation of breakage by chemical agents is associated with regions rich in heterochromatin. On this basis Loveless (1953) has suggested different mechanisms for the action of ionising radiation and of radiomimetics. The present results do not in any way support this view. Although there may well be certain differences in detail, the similarities to be found are so numerous that it would seem the mechanisms have at least some common basis.

So far we have discussed the distribution of length of acentric fragments in terms of a non-random primary breakage. It is possible that part or the whole of the observed localisation of breaks is dependent on the chance of restitution varying along the length of the chromosomes, perhaps in consequence of the polarisation from the previous

telophase. However, it is doubtful whether non-random restitution need seriously be considered as an alternative to non-random breakage since re-union of all scorable kinds occurs at such a low frequency. We have seen that the length of acentric fragments gives no indication of NSR. SR accounts for most of the scorable re-unions ; but even these number only about 10-15 per cent. of the total possible in the observable breaks. This is to be contrasted with data from the irradiation or chemical treatment of actively metabolising cells where 50 per cent. of breaks may show NSR (*e.g.* Lane, 1951 ; Darlington and Koller, 1947).

The micro-acentrics have been omitted from the above analysis. They are probably paired rings of diameter less than half of the chromatids and appear to be small interstitial deletions with a length about that of one gyre of the chromosome helix. There is a clear discontinuity in thickness between the minutes and the smallest class of acentrics considered in the above analysis. To avoid including any minutes in the distribution of X and thus overloading the class of frequencies of the shortest acentrics, all fragments less than 0·5 μ have been classified as minutes.

5. DISTRIBUTION OF BREAKS BETWEEN NUCLEI

Tables 6 and 7 give the distributions of numbers of breaks between cells of the same root tip and between root tips. These tables show that the mean number of breaks induced in dormant seeds shows great variation between seeds. The heterogeneity is much greater in the aged than in the irradiated seed. It presumably indicates that seeds differ greatly in sensitivity to ageing and to a lesser extent to irradiation. The cause of the variation may be a range in permeability governing water or oxygen content in the air-dry seeds.

As regards the distribution of breaks between cells of the same seed, if the breaks are caused by statistically independent events, these distributions for the individual root tips should be Poissonian. In view of the marked heterogeneity between tips, it is probably better to restrict the analysis to individual distributions. In the Poisson distribution, the mean and the variance are equal. The variance/mean ratio gives an efficient test of the Poissonian nature of a distribution. This test is preferable to a direct χ^2 test on the observed class frequencies as used, *e.g.* by Lea (1946), since it avoids the grouping of terminal classes and it preserves any trends in the deviations from expectation. The significance of deviations of the ratio from unity can be tested by using the ratio of the sum of squares to the mean which has a χ^2 distribution (Sukhatme, 1938).

Values of the variance/mean ratio and the significance of their deviations from unity, expressed as normal deviates, are shown in tables 6 and 7. All aged material and all but one of the irradiated seeds show significant overdispersion $V_x/\bar{x} > 1$, with deviations ranging

TABLE 6

Aged seed. Distribution of number of acentrics, N, fitted by Poisson and negative binomial. N - C_0'' per cell

Tip		0	1	2	3	4	5	6	7	8	cells n	$\frac{Vx}{\bar{x}}$	$\frac{\Sigma(x-\bar{x})^2}{\bar{x}}$	C	P	χ^2	df	P
1	Data	202	1	2	1	...	...	...	...	...	**206**	2·2	456	*9·91*	× × ×	test not applicable		
	Poisson	197·9	7·9	0·1	...	...	...	...	...	...	...	...	...	...	...			
	-ve bin.	200·83	3·51	0·99	0·37	0·16	0·07	0·03	0·01	0·03	...	...	...	...	...			
2	Data	151	10	2	1	...	...	1	...	...	**165**	2·6	429	*11·15*	× × ×			
	Poisson	143·5	20·1	1·4	0·1	...	...	...	...	...	...	...	...	...	...	10·30	1	× × ×
	-ve bin.	151·86	8·09	2·72	1·17	0·56	0·28	0·17	0·09	0·06	...	...	...	...	...	0·69	1	0·42
3	Data	268	...	1	1	...	...	...	...	...	**270**	2·4	652	*12·91*	× × ×	test not applicable		
	Poisson	264·7	5·3	0·1	...	...	...	...	...	...	...	...	...	...	...			
	-ve bin.	266·91	2·04	0·61	0·24	0·11	0·05	0·02	0·01	0·01	...	...	...	...	...			
4	Data	83	10	3	4	1	...	1	...	1	**103**	4·6	473	*16·44*	× × ×			
	Poisson	62·5	31·2	7·8	1·3	0·2	...	...	...	...	...	...	...	...	...	44·25	2	× × ×
	-ve bin.	83·94	8·77	3·87	2·17	1·33	0·86	0·56	0·39	1·11	...	...	...	...	...	2·29	2	0·32
5	Data	193	6	...	...	1	...	...	...	...	**200**	2·2	430	*9·34*	× × ×			
	Poisson	190·2	9·5	0·2	...	...	...	...	...	...	...	...	...	...	...	test not applicable		
	-ve bin.	193·47	4·48	1·25	0·46	0·19	0·08	0·04	0·02	0·01	...	...	...	...	...			
6	Data	18	10	6	1	...	2	1	...	3	**41**	6·7	267	*14·10*	× × ×			
	Poisson	56	11·1	11·1	7·4	3·7	1·5	0·5	0·1	...	...	...	...	...	...	46·39	4	× × ×
	-ve bin.	21·12	6·27	3·60	2·39	1·70	1·26	0·96	0·74	2·96	...	...	...	...	...	6·79	3	0·08
7	Data	141	18	6	4	3	1	...	1	...	**174**	2·7	461	*11·75*	× × ×			
	Poisson	116·6	46·6	9·3	1·3	0·1	...	...	...	...	...	...	...	...	...	61·33	2	× × ×
	-ve bin.	139·18	19·81	7·60	3·53	1·78	0·94	0·51	0·28	0·37	...	...	...	...	...	0·91	2	0·62
	ΣData	1056	55	20	12	5	3	3	1	4	**1159**	...	...	...	...			
	ΣPoisson	977·0	131·7	30·0	10·1	4·0	1·5	0·5	0·1	...	...	...	...	...	...	91·91	4	× × ×
	-ve bin.	1057·31	52·97	20·64	10·33	5·33	3·51	2·29	1·54	4·56	...	...	...	...	...	0·02	6	0·99

× × × significant at 0·001 level.

TABLE 7

Distribution of N fitted by Poisson and negative binomial. N - C_0'' per cell

× × × significant at 0·001 level.

TABLE 7

Fresh seed. Irradiated (2400r). Distribution of N fitted by Poisson and negative binomial. N C_0'' per cell

Tip		0	1	2	3	4	5	≥6	cells n	$V\bar{x}/\bar{x}$	$\Sigma(x-\bar{x})^2/\bar{x}$	C	P	χ^2	df	P
1	Data .	185	11	9	2	…	…	…	**207**	1·7	350	*6·10*	× × ×			
	Poisson .	174·64	29·69	2·52	0·14	…	…	…	…	…	…	…	…	38·37	1	× × ×
	-ve bin. .	182·07	18·14	4·63	1·42	0·47	0·16	0·11	…	…	…	…	…	7·01	1	0·01
2	Data .	236	10	3	2	…	1	…	**252**	2·3	581	*10·63*	× × ×			
	Poisson .	225·75	24·83	1·37	0·05	…	…	…	…	…	…	…	…	24·10	1	× × ×
	-ve bin. .	235·32	10·92	3·35	1·32	0·58	0·26	0·25	…	…	…	…	…	0·26	1	0·63
3	Data .	270	45	4	…	…	…	…	**319**	1·0	314	*0·18*	0·86			
	Poisson .	268·92	45·74	3·86	0·22	0·01	…	…	…	…	…	…	…	0·04	1	0·78
	-ve bin. .	269·93	45·39	3·54	0·17	0·01	…	…	…	…	…	…	…	…	…	…
4	Data .	189	21	5	1	…	…	…	**216**	1·3	284	*3·04*	× ×			
	Poisson .	184·06	29·45	2·36	0·13	…	…	…	…	…	…	…	…	10·73	1	× × ×
	-ve bin. .	188·42	22·48	4·06	0·82	0·17	0·04	0·01	…	…	…	…	…	0·98	1	0·59
5	Data .	172	15	5	1	…	…	…	**193**	1·4	275	*3·85*	× × ×			
	Poisson .	166·95	24·20	1·76	0·09	…	…	…	…	…	…	…	…	12·96	1	× × ×
	-ve bin. .	171·09	17·31	3·49	0·82	0·20	0·05	0·04	…	…	…	…	…	0·98	1	0·33
6	Data .	220	10	5	1	…	…	…	**236**	1·6	377	*5·76*	× × ×			
	Poisson .	213·54	21·35	1·07	0·04	…	…	…	…	…	…	…	…	27·77	1	× × ×
	-ve bin. .	218·68	13·28	2·91	0·79	0·24	0·07	0·03	…	…	…	…	…	2·33	1	0·23
7	Data .	82	16	3	2	1	…	…	**105**	1·6	171	*4·06*	× × ×			
	Poisson .	77·79	23·34	3·50	0·35	0·03	…	…	…	…	…	…	…	3·71	1	0·05
	-ve bin. .	83·02	15·35	4·44	1·44	0·49	0·17	0·09	…	…	…	…	…	…	…	…
	ΣData .	1354	128	34	9	1	1	…	**1528**	…	…	…	…			
	ΣPoisson .	1311·65	198·60	16·44	1·02	0·05	…	…	…	…	…	…	…	116·86	2	× × ×
	-ve bin. .	1348·53	142·87	26·42	6·78	2·16	0·75	0·53	…	…	…	…	…	5·16	3	0·17

× × significant at 0·005 level. × × × significant at 0·001 level.

from 3 to 16 times their standard errors. Fig. 3 shows the data graphically, the variance/mean ratio being plotted against the mean. The greatest range of acceptance for the Poisson with limits at the 5 per cent. level of significance is shown. The graph illustrates clearly the heterogeneity in mean breakage level between different seeds and the departure from the Poissonian expectation. Further, in the aged seeds there is evidence that overdispersion increases with the mean number of breaks. The trend is the more significant since cells

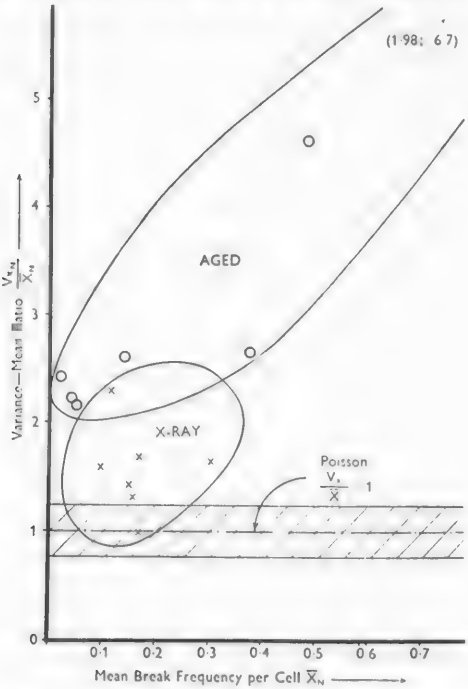

FIG. 3.—Relationship of the variance/mean ratio to the mean number of acentrics per cell showing the maximum fiducial limit (5 per cent. level) of acceptance of the Poissonian expectation ($V_x/\bar{x} = 1$).

unscorable because of high numbers of breaks are more frequent in the roots with high breakage levels. The trend towards increased overdispersion with increased numbers of breaks is not apparent in the X-rayed seed. This may be due to the lower variation in the mean breakage level. However, six of the seven roots show undoubted overdispersion.

It is surprising that this overdispersion in X-ray data has not been demonstrated by previous investigators. Lea (p. 218, 1946) gives a partial analysis of the data available at that time. He fails to include for example, data from Newcombe (1942) and Darlington and La Cour (1945). Figs. 4 and 5 show the results of an analysis of dispersion in

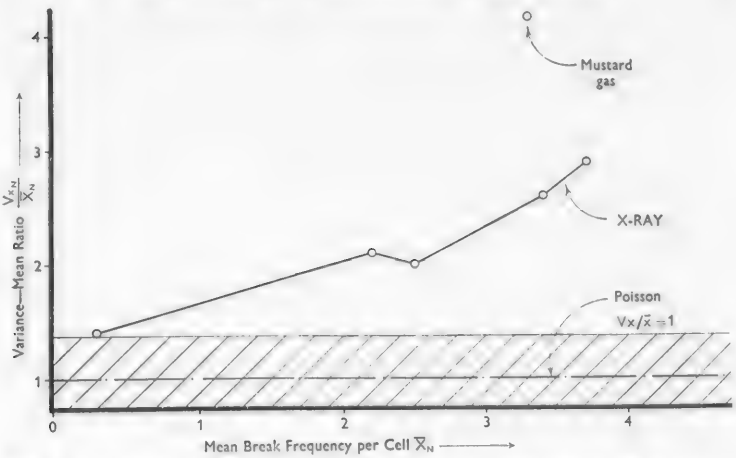

FIG. 4.—Relationship of the variance/mean ratio to the mean number of acentric fragments for X-irradiation and chemical treatment of *Tradescantia* pollen grains. Data from Darlington and La Cour (1945) and Darlington and Koller (1947).

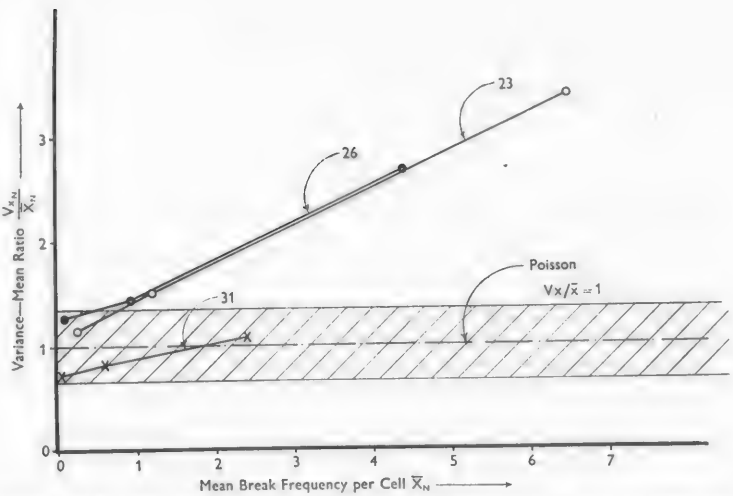

FIG. 5.—Relationship of the variance/mean ratio to the mean number of acentric fragments for X-irradiation of *Hyacinthus* pollen grains. Data from Newcombe (1942). Irradiation applied 23, 26 and 31 days before fixation.

those data. It will be seen that the degree of overdispersion is significant at high breakage levels in *Hyacinthus* and *Tradescantia* and increases with increase in the mean break frequency. In *Hyacinthus* it is clear that the degree of dispersion is also dependent on stages in cell sensitivity. The problem requires further examination. However, the analysis certainly supports the results gained in our experiments. An analysis of other published data on X-irradiation shows that most of the distributions are overdispersed. Those cases which do not depart significantly from the Poissonian form are found to have low mean break frequencies.

Few data are available for spontaneous and chemical breakage. Darlington and Upcott's (1941) data for spontaneous breakage in *Tulipa* are greatly overdispersed. Data for chemical breakage from Darlington and Koller's (1947) treatment of *Tradescantia* by sulphur mustard give a variance/mean ratio of 4·2 (C = 14·7). For comparison this is represented graphically in fig. 4 with Darlington and La Cour's (1945) irradiation data on the same material.

The Poissonian distribution is thus unsuitable for summarising data on the distribution of breaks between cells. A number of two-parameter distributions describing overdispersion in biological data has been developed (Anscombe, 1950 ; Bliss and Fisher, 1953). Of these the negative binomial is probably best because of its wide applicability and its ease of computation. The negative binomial $(q-p)^{-k}$ has two parameters, the mean m and the exponent k ; and $p = m/k$ and $q = 1+p$. The expectation of a_r, where a_r is the frequency of the rth class ($r = 0, 1, 2, \ldots$), is

$$\phi_r = \mathrm{E}\,(a_r) = \mathrm{N}\,\frac{(k+r-1)\,!}{r\,!\,(k-1)\,!}\cdot\frac{p^r}{(1+p)^{k+r}}$$

where N = total number of observations in the sample.

The equation of estimation based on the mean $pk = \bar{x} = \mathrm{S}(fx)/\mathrm{N}$ is fully efficient. The error variance of the mean is $\mathrm{V}\bar{x} = \left(\bar{x}+\frac{\bar{x}^2}{k}\right)\Big/\mathrm{N}$.

The estimation of the exponent k offers certain difficulties. Methods and their efficiencies have been discussed by Anscombe (1950) and Bliss and Fisher (1953). The small sample size hardly warrants the estimation of k by the method of maximum likelihood. The simplest method is based on the first and second moments but has an efficiency of only 60 per cent. in this particular case. A more efficient estimate can be obtained for these data using the frequency of the zero class, a_0. Since $(1+p)^k = \mathrm{N}/a_0$, an estimate of k can be found which balances the equation, $\hat{k}\log_n(1+x/\hat{k}) = \log_n(\mathrm{N}/a_0)$. Anscombe's graph shows that this estimate has an efficiency of over 98 per cent. for data of the type obtained in these experiments.

Estimates of m and k are shown in table 8. Values of a_r with the expectations ϕ_r calculated from the best-fitting negative binomial for the seven sets of data in each experiment are shown in tables 6

and 7. A comparison of a_r and ϕ_r indicates that the agreement with the negative binomial exceeds that expected purely on the introduction

TABLE 8

Statistics of the distribution of N, number of fragments per cell. Estimates of parameters and test functions for the negative binomial distribution

	Tip	n	$\bar{x}$	$\hat{k}$	U	S_U	P(U)
Aged	1	206	0·03883	0·01590	−0·04738	+0·07342	*0·52*
	2	165	0·13939	0·10490	+0·03999	+0·09311	*0·67*
	3	270	0·01851	0·00466	−0·04723	+0·07416	*0·53*
	4	103	0·48543	0·14925	+0·18797	+0·62258	*0·76*
	5	200	0·05000	0·05553	+0·01302	−0·03950	*0·74*
	6	41	1·97560	0·52991	+3·83342	+3·25923	*0·24*
	7	174	0·37931	0·19418	−0·10890	−0·70566	*0·88*
X-ray	1	207	0·16908	0·14066	−0·08545	+0·06253	*0·17*
	2	252	0·10714	0·07192	−0·01931	−0·05313	*0·72*
	3	319	0·16614	−14·22000	−0·00005	−0·56748	*0·99*
	4	216	0·15714	0·41729	−0·00912	+0·03421	*0·79*
	5	193	0·14508	0·25909	−0·01833	−0·03114	*0·56*
	6	236	0·09746	0·11240	−0·02556	+0·03782	*0·50*
	7	105	0·30476	0·61110	−0·04565	−0·06395	*0·48*

$$U = S^2-[\bar{x}+\bar{x}^2/\hat{k}]. \quad V_{(U)} = 2m(\hat{k}+1)/pq^2\left(1-\frac{R^2}{-\log_n(1-R)-R}\right) \quad N=p^4V_{\hat{k}}$$

where S^2 = variance estimate.

$$V_{\hat{k}} = \frac{(1-R)\cdot\hat{k}-1-R}{N[-\log_n(1-R)-R]^2} \quad \text{and} \quad R = \bar{x}^2/(\hat{k}-\bar{x})$$

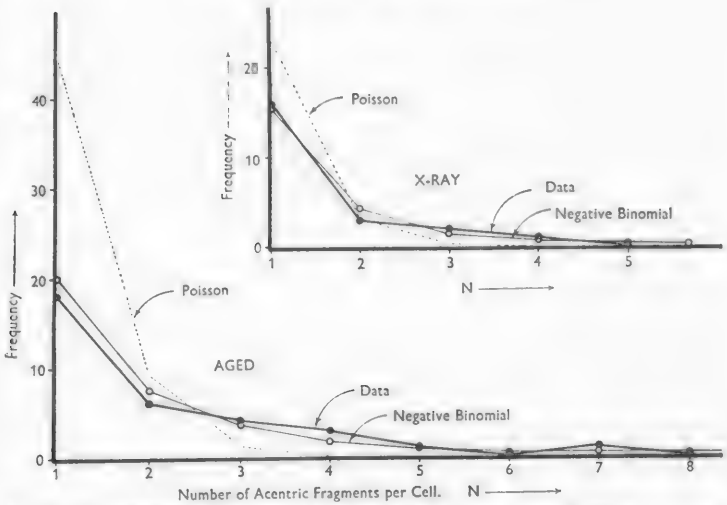

FIG. 6.—Comparison of fits obtained by the negative binomial and Poisson distributions to the data for typical root tips in aged (tip 6) and X-irradiated (tip 7) material.

of another parameter. The distribution for typical sets of data in each experiment are shown graphically in fig. 6. A satisfactory test

of agreement between a_r and ϕ_r by χ^2 is possible only in a few cases because of the low number of classes remaining after pooling to give expectations $\geqslant 5$. A less reliable set of values for χ^2 are quoted in tables 6 and 7 where low ϕ_r values are pooled so that the class expectations are $\geqslant 1$. Two efficient tests which avoid grouping of the tail frequencies have been devised by Anscombe. His test criteria, T and U, are based on differences between the observed and calculated moments. Comparison of either test function with its standard error provides a measure of agreement with the negative binomial. The difference, T, between the observed third moment and the calculated third moment is efficient when k is estimated efficiently by the moment method. The difference between the observed and calculated second moments, U, is an efficient test function when k is estimated efficiently by the method of zero class frequencies. The latter test is thus suitable for the present data. Values of U and its standard error are given in table 8. The tests show that the distributions of breaks in the cells are adequately described using the negative binomial distribution.

A negative binomial distribution can arise as the result of several quite different models which are discussed by Anscombe, and by Bliss and Fisher. They include : (*a*) inverse binomial sampling ; (*b*) heterogeneous Poisson sampling ; (*c*) randomly distributed colonies ; (*d*) immigration—birth—death process.

In the case of breakage produced by irradiation suitable arguments can be constructed from models (*b*) and (*c*) above. For spontaneous breakage, an argument based on model (*d*) is an additional possibility, but it is doubtfully distinct from (*c*). Discrimination between these and other models leading to the negative binomial can be based only on the general biological feasibility of the proposed mechanism.

Let us consider the two possible models. A negative binomial with exponent k is realised if the members of a population constitute a mixed or " compound " Poisson distribution such that the means of the individual Poisson distributions have a Type III distribution, proportional to a χ^2 distribution with $2k$ degrees of freedom.

The conditions of this model are simply met if we consider the specific breakage sensitivity (intensity), ξ, of individual cells at a fixed dose, to be a positive continuous variant. The simplest frequency distribution which ξ might be expected to follow would be the Eulerian or Pearson Type III distribution. If breakage is due to the direct action of an ion cluster the probability of obtaining N breaks in a cell with intensity ξ_i is $e^{-\xi_i}\,\xi_i^{N}/N\,!$. The distribution of N in a sample of cells where ξ_i has a Type III distribution will be a negative binomial.

While there is evidence of some variation of the sensitivity ξ in cells at different stages of development, the range of sensitivity even in a population of growing cells seems too small to result in significant overdispersion. In the population of dormant cells used in these experiments, we can assume only a very small range in sensitivity. In addition the hypothesis does not provide any explanation for the

similarity between the distribution of spontaneous and radiation-induced breaks.

As regards the second possible model a negative binomial distribution results from sampling a population whose members are grouped in colonies which are randomly distributed in some continuous dimension like space or time, and where the numbers of individuals in the colonies are distributed independently in a logarithmic distribution. This model is a simple " contagion " type model and seems to be of more general importance than models based on Neyman's or Thomas's distributions.

In a crude sense we may say that in our material " contagion " exists within cells (and possibly between cells), such that cells which suffer damage are more prone to further damage, *i.e.* a local disturbance of sufficient magnitude to cause breakage, results also in a regional instability which lowers the threshold value for further breakage in the general surroundings. An obvious source of such contagion is some diffusable chemical agent produced within the tissue. This is a concept for which there is an increasing amount of evidence in modern radiobiology.

A mechanism based on cumulative chemical action would explain the similarities which exist in the pattern of breakage produced by chemicals, by radiations and spontaneously. Similar patterns both within chromosomes and between cells could be realised if the ion clusters produced by irradiation are the centres of production of some active radicals or mutagenic substances.

A model representing this cumulative chemical effect must account for the contribution of various numbers of production centres and for the diffusion of the mutagen in time and space. As a first approximation we may utilise the number of observed breaks as a function of the number of primary centres. We may then construct a transition function for the probabilities of successive events of the form.

$$\frac{P\{N+1\}}{P\{N\}} = \frac{\alpha+N}{\beta+t}$$

where α and β are constants >0, N is the number of breaks and t is the aggregate diffusion path. This yields a probability function of obtaining N events as

$$p\{N\} = \left(\frac{\alpha}{\alpha+\xi t}\right)^{\alpha} \cdot \frac{\alpha(\alpha+1)\ldots(\alpha+N-1)}{N!} \cdot \left(\frac{\xi t}{\alpha+\xi t}\right)^{N}$$

with $\xi = \alpha/\beta$. This is a negative binomial with mean $= \xi t$ and variance $= \xi t+(\xi t)^2/\alpha$. So that $m = \alpha t/\beta$, $k = \alpha$, $p = t/\beta$ and $q = 1+t/\beta$ and the distribution function is identical with that obtained from the model based on heterogeneous Poisson sampling.

It is perhaps worth mentioning briefly at this stage a closely related model which may apply. If the initial events are distributed at random but the derived substances obey a geometrical distribution

as opposed to the logarithmic form in the above model, the resulting distribution will be a Polya distribution (Anscombe, 1950). In the absence of very extensive data, discrimination between the negative binomial and the Polya would be extremely difficult. In any case such minor variations are unimportant at this juncture when the main consideration lies in the choice between a direct or an indirect action.

6. CUMULATIVE ACTION

The concept of cumulative action is based on the existence of intermediate chemical steps between the primary physical events of X-irradiation and final breakage. It thus removes the gross simplifications of the target theory and explains immediately the many similarities in effects and distributions of damage obtained after treatment of chromosomes by such different agents as ageing, irradiation and chemical mutagens.

The target theory can be considered to be the limiting case which is approached when interaction between ion clusters is reduced by isolating them in time or space. Under conditions of isolation the probability of reaching critical concentrations of active mutagen will be limited to the immediate proximity of the primary ion clusters and particular breaks will be ascribable to particular particles as demanded by the target theory. This appears to be the case at low doses or at very low intensities. However, there is increasing evidence, some of which has been given above, that at high doses serious deviations from the expectations of the target theory occur.

These problems have been investigated in three ways: (1) by altering the total dose keeping intensity constant, (2) by altering the intensity at which a constant dose is given and (3) by fractionating a constant dose. The target theory would predict that the efficiency of breakage per unit dose should remain constant under all these conditions. Any cumulative effect would show by departures from linearity under (1) and by an increased number of breaks per unit dose at higher intensities or without fractionation under (2) and (3). Where deviations from the expectations of the target theory occur they are always of the type predicted by the hypothesis of cumulative action. There is, however, great controversy in the interpretation of these deviations, various authors accounting for them in terms of non-random re-union, changes in rates of re-union under different physiological conditions, etc. It would seem that greater consideration should be given to explaining all the results in terms of cumulative action.

In particular more experimental work is needed on the distribution of breaks between cells at high intensities and doses. Cumulative action leading to a negative binomial distribution predicts that the variance should exceed the mean by a quantity approximately equal

to $\frac{\bar{x}^2}{k}$. We have seen that in some data analysed here (figs. 4-6) this expectation is realised. In other investigations, *e.g.* Sparrow (1951), complex changes in nuclear sensitivity apparently cause both mean and the " contagion factor ", k, to undergo changes.

There are two further aspects of irradiation work which require discussion in terms of cumulative action. They are the effect of oxygen and of ion density. The interaction of oxygen with X-irradiation indicates that irradiation initiates some chemical chain-reaction involving oxygen. The type of compounds concerned with breakage has not yet been determined but they are probably peroxy and epoxy compounds (Read, 1951 ; Phillips, 1956). These chain-reactions, which can be interrupted in various ways, provide the right chemical models for cumulative action. As regards ion density, it has been demonstrated that the yield of peroxide increases with oxygen for low ion density radiation but for high ion density radiation, *e.g.* α-rays, oxygen has no such effect, the products being localised to the track of the particle. It is hoped to investigate experimentally the effects of some of these variables on overdispersion.

7. SUMMARY

1. A comparison of chromosome breakage in *Allium cepa* induced by ageing (4 year storage) and by X-irradiation (2·4 Kr) of the dry seed shows great similarity in pattern.

2. The types of aberration produced are the same in the two treatments. Excluding minutes, 98 per cent. of all aberrations are simple terminal deletions. Chromatid breakage appears to be absent and non-sister re-union rare.

3. The distribution of breaks within the chromosomes was studied by determining the frequency distribution of length of acentric fragments. Near-terminal regions of the chromosome arm are very susceptible to damage ; centromere regions are susceptible but less so ; mid-arm regions are resistant. The distributions of breaks within chromosomes are very similar in the two treatments.

4. The absence of correlation between the length of acentric fragments and the number induced in the cell indicates a random or near-random distribution of damage between chromosome arms after both treatments.

5. The distribution of breaks between cells of the one seed shows overdispersion as compared with the Poisson distribution. Overdispersion is greater after ageing than after X-irradiation for the same mean number of breaks. The overdispersed distributions obtained after both treatments can be fitted with a high degree of efficiency by the negative binomial.

6. Analysis of previously published data shows that overdispersion is frequent in breaks induced by X-irradiation, by chemical mutagens

and spontaneously. In general, overdispersion increases as the mean number of breaks increases.

7. It is suggested that these similarities in pattern of breakage induced by very different agents indicate a similar chemical basis for the induction of breakage. In particular, the evidence indicates that the initial sites of ionisation after X-irradiation interact, presumably by the production of mutagenic substances or radicals which have a definite half-life within the cell, during which time they can diffuse in both time and space. A "contagion" model based on cumulative action is proposed to explain the negative binomial distribution.

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ADVANTAGE OF THE WHITE EYE MUTANT OF *DROSOPHILA MELANOGASTER* OVER THE WILD TYPE IN AN ARTIFICIAL ENVIRONMENT

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1. INTRODUCTION

Most mutants of *Drosophila melanogaster* are at a disadvantage compared with the wild type in environmental conditions provided by nature. It is a truism of genetics to state that the wild type gene is so because mutative deviants cannot compete with it. For this reason the mutant types of the laboratory are infrequent in nature. In some Drosophila experiments of Gordon (1935), ebony and wild type flies were simultaneously released in a locality in which neither form was present. The frequency of ebony rapidly decreased in five to six generations from 50 to 11 per cent. Gordon believed that the heterozygote as well as the recessive was at a disadvantage compared with the wild type.

If, however, the environment is changed so that it is novel both for the wild type and the mutant, it is conceivable that some specific environment might enable a mutant allele to be at an advantage compared with the wild type. It is even possible to imagine selective rates of multiplication of flies possessing wild type and mutant alleles respectively to be so altered that the latter would either replace the former, or cause a new system of gene frequencies to be set up. This would occur if certain features of the environment favoured the wild type and the mutant at different times.

Different genotypes probably always have different reproductive rates over the whole or part of the environmental spectrum found in nature. It is therefore important to explore the environmental contexts in which genotypes differing mainly in a single allele manifest different types of behaviour affecting their reproductive rates. If different types of environmental reaction are inherited and unusual environments are differentially sought, a new equilibrium will be set up between mutant and wild type.

The general object of the experiments described here was to see whether an environment can be devised in which the white eye mutant of *Drosophila melanogaster* would be at an advantage compared with the wild type.

2. PRELIMINARY EXPERIMENTS

The idea underlying the experimental work was to see if selection effects for migratory or olfactory reaction could be detected under

conditions in which flies would experience difficulty in finding food.

Various kinds of apparatus were considered, among them the complicated types devised by McIndoo (1926, 1933) for testing olfactory discrimination in insects. All these were rejected in favour of a simpler form of apparatus which would serve to expose the differential migratory capacities and olfactory reactions of wild type and mutant.

The apparatus

The apparatus used consisted of three half-pint milk bottles with rubber bungs connected by 4 mm. bore glass tubing. There were two leads of similar length (50 cm.) from the central bottle A, one connected with C an empty bottle and the other connected with B, a bottle containing the standard food of conventional type, in which to prevent mould growth, the yeast was a propionic acid resistant strain. All glass tubing was covered with black insulating tape to exclude light.

The three bottles were each enclosed in lightproof cardboard boxes, cotton wool being packed round the tubing where it entered the boxes. The flies were thus in complete darkness throughout the period of the experiment.

Preliminary experiment 1

The preliminary experiment which led to the extended use of this apparatus may now be described.

A mixture of 224 red-eyed flies from a mixture of several geographical strains (hereinafter known as G.S.) kept in mass culture for several generations together with 6 white-eyed flies of diverse origin, were placed in bottle A. After 24 hours the flies in the three bottles were counted and classified.

The results were :

Bottle A (the original bottle) 74 red females, 93 red males, 1 white male.

Bottle B (with food) 13 red females, 1 red male, 2 white females, and 3 white males.

Bottle C (empty) 23 red females, and 20 red males.

The striking feature of this experiment was that of 6 white-eyed flies, 5 had migrated to the food bottle, indicating a tendency to a high proportional migration to food of the white mutant compared with the wild type.

It was at this point that the experimental material took the form of a mixture of equal numbers of red-eyed females, red-eyed males, white-eyed females and white-eyed males. These, placed in the

central bottle, were given equal opportunities of migration in the dark to another empty bottle or to a bottle containing food.

Although little seems to be known about the migratory propensities of flies in light and darkness respectively, it was considered desirable to conduct all further experiments in the dark. The reasons for this decision were :

1. The difficulty of equal illumination for all parts of the apparatus.
2. Variations in natural daylight might differentially affect mutant and wild type.
3. The difficulty of satisfactory lighting at night.

In experiments conducted in daylight, flies reaching the rubber connections joining the glass tubing turned round and went back. It was also observed that flies were deterred from entering the dark regions where the tubes passed through the rubber bungs.

Preliminary experiment 2

The experiment was set up to reveal the relative ability of males and females of both red- and white-eyed stocks to find a bottle containing food in a dark room at a controlled temperature of 25° C. A half-pint milk bottle containing the normal propionic acid food medium was placed in the middle of the room. Two hundred flies were released simultaneously in the dark room, as far away as possible from the food bottle. They comprised 50 red females and 50 red males from the G.S. stock, and 50 white females and 50 white males from the Cambridge white stock. The flies had been without food and water for two hours before release.

Every half hour the food bottle was replaced by a fresh one. After 6 hours the flies caught in the 12 food bottles comprised 22 red-eyed females, 14 red-eyed males, but no white-eyed flies.

It seems that under these special conditions in which the flies were able to migrate freely, the reds were superior to the whites in their ability to find food.

3. THE MAIN EXPERIMENTS

In all the following experiments the 3-bottle apparatus was used.

Material

The flies used consisted of the following races of *Drosophila melanogaster*.

(1) Cambridge mass culture white. This strain was considerably inbred but may have been somewhat heterogeneous.
(2) A mixture of ten geographical strains of wild type red-eyed (G.S.).
(3) Heterozygous red-eyed females—the F_1 of the cross G.S. red females by white Cambridge males.
(4) Samarkand red-eyed, from an inbred stock kept going by sister-brother mating for 286 generations.
(5) Lima inbred red-eyed, inbred by sister-brother mating for 36 generations.
(6) The F_1 hybrid Samarkand female by Lima male.

At the start of each trial with the apparatus previously described, the flies to be tested were placed in bottle A, and counted 2-3 hours before the beginning of the trial to enable them to recover from the effects of etherisation. Trials were started at about 4 p.m. and 24 hours later the classification of the flies for sex and eye colour was effected.

The food medium. This consisted of the following : 108 gm. maize meal ; 12 gm. agar powder ; 77 c.c. black treacle ; 520 c.c. water. Six c.c. of propionic acid yeast suspension was added to each bottle of the prepared food.

The experimental results

Experiment 1. To observe the migration from A to B (food) and to C (no food) of red-eyed females (50), red-eyed males (50), white-eyed females (50) and white-eyed males (50).

The results are given in table 1.

TABLE 1

Composition of flies which migrated to B (food) and C (no food) in trials with each of two apparatuses, Nos. 1 and 2. G.S. reds and Cambridge whites

No. of the apparatus	Red females	Red males	White females	White males	Total	No. of trials
2 B	224	281	543	570	1618	33
2 C	70	73	70	97	310	
1 B	41	84	99	116	340	20
1 C	55	52	44	7	229	

The results of the 33 trials with apparatus 2, clearly show that whites are about twice as efficient as reds in their ability to migrate to food, and that the sexes are somewhat similar in their response. The males migrate slightly more than the females though the difference is not statistically significant.

The results from bottle C (no food) indicate no clear differences between reds and whites, or between males and females in each eye-colour class. Comparing migration to food and no food, 1618 flies go to the food bottle and only 310 to the no-food bottle. It seems evident that an olfactory response is involved.

The results with apparatus 1 again indicate that whites (215) go to food more often than reds (125). The ratio of 1·72 : 1·00 in favour of whites may be compared with the ratio of 2·20 : 1·00 obtained with apparatus 2.

To be noted is the fact that the type which migrated most was the white male. Noteworthy also was the greatly fluctuating behaviour of the flies in the different trials. The results of single trials are capricious, and it is only when several trials are pooled that the advantage of white is clearly marked. Variation between trials cannot be accounted for by any environmental factor that can be postulated.

To illustrate the range of variation between the results of trials, a few may be given in detail (for bottle B food) :

Classes	Trial number								
	10	14	19	20	33	23	24	21	29
Red female . .	0	0	0	0	0	27	24	17	17
Red male . .	7	0	4	0	9	14	22	13	21
White female .	37	37	0	5	12	38	30	23	28
White male . .	30	40	5	7	10	30	34	10	25

Trial 14 is of special interest, in that no reds went to food at all, whereas 77 whites did so.

Experiment 2. In this experiment heterozygous red-eyed flies from the cross G.S. reds by Cambridge whites replaced the homozygous reds used in Experiment 1.

The results are given in table 2.

TABLE 2

Composition of flies which migrated to B (food) and C (no food) in trials with each of the two apparatuses Nos. 1 and 2, heterozygous G.S. reds and Cambridge whites

No. of the apparatus	Red females	Red males	White females	White males	Total	No. of trials
2 B	12	71	58	92	233	15
2 C	11	138	21	36	206	
1 B	23	133	75	98	329	15
1 C	7	46	11	9	73	

The main points of importance in table 2 are as follows :

Apparatus 2.

1. The number of flies migrating to food (B) is only slightly greater than the number which migrated to no food (C). (233 : 206).

2. The flies which migrated to food consisted of 150 whites and 83 reds, and the advantage of white over red is again obvious.

3. The migrating capacity of heterozygous reds where four types are present is very low. Under these particular experimental conditions, the substitution of heterozygous red females for homozygous was not advantageous but rather the reverse.

4. The proportion of males to females migrating to both food and no food differed significantly from equality. For food the ratio was 163 males to 70 females, and for no food 174 males to 32 females.

5. In flies which migrated to no food the advantage was with red and not with white. This is chiefly due to the large excess of red

males which considerably outnumbered the total of the other three classes (139 red males to 63—the rest).

Considering the results of apparatus 1, the main features are as follows :

1. The number of flies migrating to food is much greater than the number which went to no food (329 : 73). The olfactory reaction came into play in this experiment, although with apparatus 2 it was only slight.

TABLE 3

Composition of flies which migrated to B (food) and C (no food) in trials with five apparatuses. Material—Lima inbred reds and Cambridge whites

No. of the apparatus	Red females	Red males	White females	White males	Total	No. of trials
2 B	87	101	195	214	597	15
2 C	4	6	8	8	26	
1 B	3	9	34	94	140	15
1 C	0	3	12	61	76	
3 B	1	2	56	56	115	5
3 C	0	1	8	7	16	
4 B	9	11	57	60	137	5
4 C	2	2	7	14	25	
5 B	39	37	76	63	215	5
5 C	18	20	30	42	110	

2. The flies which migrated to food consisted of 173 whites to 156 reds. The advantage of white over red was small. The red-eyed males once more constituted the largest class.

3. Only 73 flies migrated to no food, the red-eyed males again being the largest class. Here there was no advantage of white over red, but rather the reverse (53 red to 20 white).

Experiment 3. In this experiment, the inbred line of Lima (50/11), replaced the G.S. reds of Experiment 1. In addition to 15 trials with apparatuses 1 and 2, 5 trials were made with each of three more apparatuses.

The results of this experiment are given in table 3.

In these experiments the advantage of white over red in the capacity to go to the food bottle was very great, the numbers for the 5 apparatuses being 319 red to 905 white.

It should be remembered, however, that inbreeding of the Lima strain must certainly have caused some depression in vigour and thus

the relative advantage of white is probably not due to the simple monogenic difference between the stocks but to inbreeding depression.

TABLE 4

Composition of flies which migrated to B (food) and C (no food) in trials with three apparatuses. Material—Samarkand inbred reds and Cambridge whites

No. of the apparatus	Red females	Red males	White females	White males	Total	No. of trials
3 B	6	26	203	232	467	13
3 C	0	0	4	5	9	
4 B	1	6	127	146	280	13
4 C	0	0	3	4	7	
5 B	14	26	243	227	510	13
5 C	0	2	10	17	29	

Experiment 4. This experiment was similar to Experiment 3, except that the mixed G.S. reds were replaced by Samarkand inbreds. The Samarkand line had been inbred for 286 generations. The results are given in table 4.

TABLE 5

Composition of the flies which migrated to B (food) and C (no food) in trials with three apparatuses. Material—hybrid Samarkand × Lima, and Cambridge whites

No. of the apparatus	Red females	Red males	White females	White males	Total	No. of trials
3 B	11	41	61	53	166	5
3 C	0	0	5	9	14	
4 B	1	4	14	16	35	5
4 C	0	3	4	7	14	
5 B	23	61	77	77	238	5
5 C	0	5	13	23	41	

These results are similar to those of Experiment 3. The activity of the Samarkand strain is very low, being even less than that of the Lima inbreds. The comparison here is rather one of lack of vigour through inbreeding, and the great difference in activity between red and white has probably no bearing on the main object of the experiments.

Experiment 5. In this experiment the red-eyed flies consisted of the F_1 of the cross Samarkand × Lima—the two highly inbred strains. The results are given in table 5.

These results indicate that the first generation hybrid between Samarkand and Lima is more active than either of the two long inbred parent strains. The increased activity may be ascribed to heterosis. The presumed heterotic condition raises the activity of

TABLE 6

Migration to food of reds of different origin compared with standard Cambridge white

Source of reds	Number going to food		Ratio
	White	Red	White to red
Geographical races . .	1127	630	2·1 : 1·0
Lima inbred . . .	995	299	3·0 : 1·0
Samarkand inbred . .	1178	79	14·9 : 1·0
F_1 Samarkand × Lima .	298	141	2·1 : 1·0

the F_1 to a level comparable with that of the G.S. stock. In support of the main conclusion from Experiment 1 that under these experimental conditions white has an advantage over red, it is important to note that even with maximum heterotic vigour of the reds, there is still a 2 to 1 advantage in favour of white.

TABLE 7

Classification of red females into homozygous and heterozygous migrating to food and no food respectively

No. of the apparatus	Heterozygous	Homozygous	No. of trials
2 B	224	131	20
2 C	66	51	
1 B	129	68	20
1 C	51	16	

A summarised comparison of the behaviour of various reds with Cambridge white is given above in table 6.

Experiment 6. Although in Experiment 2 the substitution of red-eyed heterozygous females for homozygous ones did not point to an advantage in favour of the heterozygous form, but rather the contrary, it was decided to compare directly the behaviour of heterozygous and homozygous reds, using females only.

Homozygous reds came from the G.S. stock. Heterozygous reds came from a mating of 5 G.S. red females and 5 Cambridge white males. The number of flies which took part in each trial was 50, consisting of 25 homozygous and 25 heterozygous flies.

Migrating flies were classified into heterozygotes or homozygotes by means of a test mating with Cambridge white males, three of which were confined with each female to ensure offspring. The results are given in table 7.

From this table it is clear that :

1. Heterozygotes migrating to food considerably outnumber homozygotes, the ratio being 1·7 : 1·0 in apparatus 2 and 1·9 : 1·0 in apparatus 1.

2. In flies migrating to no food there was a slight advantage (66 : 51) of the heterozygote in apparatus 2 and a much greater advantage in apparatus 1 (51 : 16).

4. DISCUSSION

It is evident from the results of the experiments that migratory movement *per se* is distinct from movement due to olfactory response. Olfactory response is the reaction of the flies to the stimulus of food. Migratory behaviour is no doubt due to interaction of genotypes of different degrees of vitality, with the general environment, and with other factors conditioning chance restlessness of a random nature. Such environmental factors as temperature and humidity seem to play little part.

The sum total of causes promoting mobility causes great and uncontrollable variation between trials.

The results in every experiment showed that more flies migrated to food than to no food, so that some sort of olfactory response is involved. Olfactory reaction is thus superimposed on capricious mobility. Flies going to food will do so for more than one reason. All will have migratory capacity. Some will reach food by pure chance. Others will have purposively responded to the stimulus of food. Chance meandering probably accounts for the flies going to the empty bottle.

In most experiments, males were generally more mobile than females, though the advantage of males over females was not always statistically significant.

Migratory behaviour of red-eyed males and females tends to be similar, as is also the behaviour of white-eyed members of both sexes. The behaviour of the reds is largely independent of the whites.

The migratory activity of the standard Cambridge white stock is about twice as great as that of the G.S. red-eyed stock. Inbreeding by prolonged sister-brother mating reduces greatly the migratory activity of red-eyed flies of the two inbred stocks, Lima and Samarkand. The inbred Lima line has only one-third and the inbred Samarkand only about one-fifteenth of the migratory activity of the Cambridge whites. The difference in migratory capacity between the two inbred stocks may be due either to genetical factors for vigour, or to a more prolonged period of inbreeding in the Samarkand stock.

The cross between Samarkand and Lima stocks is more active than either parent and is in fact as active as the extremely heterozygous mixed geographical race. It seems that heterosis augments both migratory and olfactory reaction but even with the maximum heterotic effect obtainable, the whites have a 2 to 1 advantage over the reds.

A possible criticism is that the genetical background in which the white mutant is manifested, might have imparted a greater degree of vigour to this race, and that the genetical background and not the white gene was the predominant factor in causing superior mobility and olfactory response. This is unlikely, because various genotypes of red-eyed flies were used, and none, even the most heterotic, had a migratory capacity equal to the Cambridge whites.

In a direct comparison between reds of the G.S. homozygous and heterozygous red-eyed females (G.S. × Cambridge white), with no males present, the heterozygotes had a greater migratory capacity and a greater olfactory response. But when heterozygotes were substituted for homozygotes in the presence of male and female whites they seemed to be at a disadvantage compared with homozygous females under similar conditions.

5. SUMMARY

Under the special environmental conditions described here, and novel to both the red-eyed wild type and the mutant white Cambridge stock of *Drosophila melanogaster*, the white is at an advantage, and the heterozygous red-eyed females may have an advantage over homozygotes.

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NEW EVIDENCE OF HETEROSIS IN NATURALLY OCCURRING INVERSION HETEROZYGOTES IN *DROSOPHILA PSEUDOOBSCURA*

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1. REVIEW OF THE PROBLEM

THE occurrence in natural populations of *Drosophila* of inversion heterozygotes has long been known. Positive evidence that this chromosomal polymorphism has an adaptive function came with the finding that the frequencies of certain gene arrangements in some populations of *Drosophila pseudoobscura* undergo cyclic changes which follow the annual succession of seasons (Dobzhansky, 1943). Wright and Dobzhansky (1946) then made experimental populations in which progenies of flies collected in nature were allowed to breed for a series of generations in population cages. The proportions of chromosomes with different gene arrangements were observed to change from generation to generation. In populations of uniform geographic origin (*i.e.* composed of progenies of flies collected in the same locality) the changes usually obeyed a simple rule. Apparently stable equilibria became established, at which the chromosomes with different gene arrangements continued to occur in the populations. This behaviour is consistent with the hypothesis that the chromosomal polymorphism is balanced ; the inversion heterozygotes are heterotic, and the corresponding homozygotes are adaptively inferior to the heterozygotes. Later it was found (Dobzhansky, 1950) that in geographically mixed populations (derived from flies collected in different localities) heterosis is no longer the rule ; the heterozygotes may be equal, superior, or inferior to the homozygotes.

The hypothesis of heterosis was verified both in experimental and in natural populations. Dobzhansky (1947) compared the frequencies of inversion homo- and heterozygotes among individuals grown under optimal conditions with those among flies which developed in severely crowded population cages. The frequencies obeyed the binomial square rule among the former, but among the latter there was an excess of heterozygotes and a deficiency of homozygotes. Dobzhansky and Levene (1948) then compared the frequencies of inversion homo- and heterozygotes among eggs deposited by wild-caught females of *Drosophila pseudoobscura*, and among adult males of the same species

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collected in their natural habitats. The binomial square rule was obeyed quite well in the egg samples. Among the wild-caught males, the deviations from the proportions demanded by the binomial square rule were not statistically significant in most samples. But the combined data, for males collected in 22 localities mostly in California, showed a quite significant excess of heterozygotes. This is as expected if the inversion heterozygotes are favoured by differential survival in most populations most of the time.

Da Cunha (1953) found some natural populations of *Drosophila willistoni* in which more than 50 per cent. of the females are heterozygous for certain inversions in the X-chromosomes. To be sure, frequencies of heterozygotes in excess of 50 per cent. may be observed without heterosis in populations which are in the throes of rapid genetic changes, and which therefore are not at equilibrium. This difficulty was removed when da Cunha (1956) found several inversions which maintained frequencies of heterozygotes in excess of 50 per cent. in experimental populations of *D. willistoni*, even after these populations reached stable genetic equilibria. Dobzhansky and Pavlovsky (1955) described a population of *D. tropicalis* from a locality in Central America in which about 70 per cent. of the individuals were heterozygous for a certain inversion. An experimental laboratory population derived from the flies from this locality contained 96 per cent. of heterozygotes for the same inversion. Pavan, Dobzhansky, and da Cunha (1957) observed in a locality in Peru a population of *D. paulistorum* with 79 per cent. of heterozygotes for a certain inversion. A somewhat lower frequency, about 60 per cent., of heterozygotes for another inversion was encountered in *D. willistoni* in north-eastern Brazil. The interest of this case is, however, heightened by the fact that the excess of heterozygotes was met with not in one but in several localities, the most remote ones separated by a distance of at least 200 kilometres, and in one of these localities the excess was ascertained at different seasons of the year.

It would certainly be wrong to conclude, as some authors did from the above data, that excesses of heterozygotes must be present at all times in all populations. The occurrence in some natural populations of rapid seasonal changes in the frequencies of chromosomes with different gene arrangements is in itself evidence that the relative fitness of at least some inversion homo- and heterozygotes varies in different environments. An extraordinary sensitivity to environmental changes was already found in *Drosophila pseudoobscura* by Wright and Dobzhansky (1946). At 25° C., the fitness of a certain inversion heterozygote was more than double that of a certain homozygote, while at 16° C. both were apparently alike in fitness. Conversely, certain karyotypes in *D. persimilis* were about equal in fitness at 25° but very different at 16°. In this connection, it is probably not accidental that *D. pseudoobscura* is more at home in warmer and *D. persimilis* in cooler habitats. Da Cunha (1951) and Dobzhansky and

Spassky (1954) induced changes in the relative adaptive values of heterozygotes and homozygotes for some inversions in *D. pseudoobscura* by feeding experimental populations on different species of yeasts. When fed on a nutrient medium with a certain species of yeast, a normally heterotic inversion heterozygote became adaptively inferior to one of the homozygotes at 21°, although not at 25° nor at 16°. As a consequence, stable genetic equilibria were established in some but not in other experimental populations. Yet, no differences in the behaviour of experimental populations of *D. willistoni* fed on different species of yeasts were detected by da Cunha (1956).

Perhaps the most striking demonstration of a delicate sensitivity of inversion homo- and heterozygotes in *Drosophila pseudoobscura* to the environment was adduced in experimental populations in which as many as six different karyotypes were simultaneously present (Levene, Pavlovsky, Dobzhansky, 1954). The adaptive value of a karyotype depends not only on such variables as temperature and food but also on what other karyotypes are living in the same medium. Thus, A may be superior to B in the absence of C, but it may become inferior to B when C is present. A different kind of sensitivity was discovered in *D. robusta* (Levitan, 1951, 1954, 1955). The gene arrangements in the two limbs of a metacentric chromosome show, in at least some populations of this species, a non-random association, which suggests that the chromosome acts as an organised unit rather than as a mechanical aggregate of genes. Moreover, this non-randomness is more pronounced in males than in females ; seasonal changes in the frequencies of the gene arrangements are also more evident in the male than in the female sex. It should also be noted that in *D. pseudoobscura* and in *D. persimilis* seasonal changes occur in populations of some geographic localities but not in others (Dobzhansky, 1956 and older work). It certainly does not follow that the carriers of the different karyotypes are differentiated in fitness in the former but not in the latter localities.

The purpose of the present article is to report some new, and quite uncomplicated, evidence that the heterozygotes for chromosomal inversions naturally occurring in *Drosophila pseudoobscura* and *Drosophila persimilis* are indeed superior in fitness to the corresponding homozygotes.

2. THE DATA

Strains of *Drosophila pseudoobscura* and *D. persimilis* have been maintained for a number of years in our laboratory. With rare exceptions, each strain is derived from a single fertilised female collected in the natural habitats of the respective species, mostly by one of the authors (Th. D.). The dates of collecting have been recorded. The chromosomal constitution which these strains possessed soon after their establishment in the laboratory is also recorded on the basis of the examination of the giant chromosomes in the larval salivary

glands. Some of the strains carried one or more kinds of heterozygotes for inversions in the third chromosomes ; this is, of course, as expected, since at the start each strain must have contained at least four third chromosomes, two derived from the original female, and two from the original male progenitor. The question, then, is this : Does the chromosomal polymorphism persist in the laboratory strains for as long or longer than it may be expected to persist owing to chance alone ?

Laboratory strains are maintained by periodic transfer of samples of flies from the old culture bottles to fresh cultures ; therefore, the unfixed genetic variants in each strain are exposed in every generation to the risk of not being included among the parents of the succeeding generation. If these variants are adaptively neutral, the risk is a function of the number of flies of the preceding generation which become the parents of the succeeding generation. This number can be only approximately estimated, because for maintenance of routine laboratory stocks the flies are transferred without being counted. Mr B. Spassky, who has maintained most of these stocks during most of the time which they have been kept in this laboratory, estimates that the average numbers of the flies transferred from culture to culture were close to twenty, and the maximum numbers close to forty, in each case about equally divided between females and males. For our present purposes an overestimate of this number is preferable to an underestimate ; we shall, therefore, use both the probable and the upper estimates in the calculations. The strains are normally kept in a constant temperature room at about 16° C., but most of them were kept for shorter or longer periods at room temperatures, especially during the early years. They are transferred to fresh cultures about once in six weeks. We shall use six weeks as the estimate of the length of a generation, although when the strains were kept at room temperatures they had to be transferred at shorter intervals.

In November 1956 samples of eggs deposited by the flies from the different strains were taken, and placed in culture bottles in a way to avoid overpopulation and crowding of the larvæ. Salivary glands from about ten larvæ from each strain, one gland per larva, were stained in acetic orcein. The preparations were made by Mrs N. Spassky ; the gene arrangements in the third chromosomes were examined and recorded by one of us (Th. D.). The results are summarised in table 1.

Table 1 lists 28 strains of *Drosophila pseudoobscura* and 11 strains of *D. persimilis*. For each strain are given the name of the locality in which the wild progenitor was collected, the date of collecting, an estimate of the number of generations during which the strain was kept in the laboratory, and the gene arrangements in the third chromosomes which were found in November of 1956. For descriptions of these gene arrangements, and for drawings of the chromosome configurations in some of the inversion heterozygotes, see Dobzhansky

(1944). In only three strains, marked in table 1 by asterisks, have the results of the examination in 1956 deviated from those of the

TABLE 1

The gene arrangements in the third chromosomes in laboratory strains of Drosophila pseudoobscura *and* Drosophila persimilis

Geographic origin	Collected	Generations	Chromosomes
D. pseudoobscura			
Alturas, Calif.	June 1940	143	AR
Amecameca, Mexico	March 1938	166	TL*
Atitlan, Guatemala	Feb. 1928	167	TL
Black Mesa, Ariz.	June 1940	143	AR
Chichicastenango, Guat.	Feb. 1938	167	TL, SC
Coffee Creek, Calif.	July 1940	142	ST, AR
Cuernavaca, Mex.	March 1928	166	CU, TL
Estes Park, Colo.	Aug. 1941	132	EP, TL, PP
Flagstaff, Ariz.	June 1940	143	AR
Huehuetenango, Guat.	Feb. 1938	167	TL*
La Grande, Wash.	Aug. 1932	211	AR, ST
Mara Lake, B. Col.	Aug. 1934	193	AR, ST
Mariposa, Cal.	July 1940	142	AR
Methow, Wash.	June 1940	143	AR, ST
Morelia, Mex.	March 1938	166	SC, TL
Oaxaca, Mex.	Sept. 1935	184	CU, OX
Orizaba, Mex.	March 1938	166	CU, TL
Pachuca, Mex.	March 1928	166	TL
Patzcuaro, Mex.	March 1938	166	SC, TL
Perpetua, Ore.	June 1940	143	AR, ST
Pikes Peak, Colo.	Aug. 1941	132	AR, PP
St Helena, Calif.	July 1940	142	AR, ST
Seattle, Wash.	Aug. 1932	211	ST
Tehuacan, Mex.	March 1938	166	EP, TL
Totonicapan, Guat.	Feb. 1938	167	CU, SC
Whitman N F., Ore.	June 1940	143	AR, ST
Yollo Bolly, Calif.	July 1940	142	AR, ST
Yuma, Ariz.	Apr. 1941	130	AR, ST
D. persimilis			
Coffee Creek, Calif.	July 1940	142	KL, MD
Deer Creek, Calif.	July 1940	142	KL, WT
Hope, B. Col.	Aug. 1934	193	KL
Nojogui, Calif.	? 1934	192 (?)	WT
Orick, Calif.	July 1940	142	KL, MD
Quesnel, B. Col.	Aug. 1934	193	KL
St Helena, Calif.	July 1940	142	KL, MD
Sequoia N. P., Calif.	Aug. 1940	141	ST
Stony Creek, Calif.	July 1940	142	ST, KL
Weott, Calif.	July 1940	142	KL*
Yollo Bolly, Calif.	July 1940	142	MD

AR—Arrowhead, CU—Cuernavaca, EP—Estes Park, KL—Klamath, MD—Mendocino, OX—Oaxaca, PP—Pikes Peak, SC—Santa Cruz, ST—Standard, TL—Tree Line, WT—Whiteney.

original examination. In all three cases, two different arrangements were originally found but only one was still extant in 1956. More precisely, the Amecameca, Mexico, strain contained in 1938 CU as well as TL chromosomes, but only TL has been preserved. In the Huehuetenango, Guatemala, strain TL and SC chromosomes were present in 1938, but SC was lost. In the Weott, California, strain

of *Drosophila persimilis* KL and MD chromosomes were found in 1940, and only KL in 1956. In 12 strains, only a single third chromosome gene arrangement was found at the original examination, and the same chromosome structure was seen again in 1956. In 23 strains, two different third chromosomes were originally found, and the same two found again in 1956; and in 1 strain three different third chromosomes were found and recovered again. Most of the strains have, evidently, maintained their chromosomal composition during more than 100 generations which they spent in the laboratory cultures.

3. MATHEMATICAL ANALYSIS

Sewall Wright (1931) investigated inbreeding in a random mating population. If the population consists of N_m males and N_f females the effective population size, N, is defined by $1/N = 1/(4N_m)+1/(4N_f)$. If $N_m = N_f$, $N = N_m+N_f$. In the present material, each culture was started with N′ flies chosen at random from the previous culture. Thus N_m fluctuated at random about a mean value of N′/2, and because the sexes were not precisely equally frequent, the effective population size over a period of time is close to N′−1. Since N′ is only known very roughly, we can take N = N′ with no serious error.

Wright (1931) gave a recurrence relation for the coefficient of inbreeding F, and for the panmictic index P = 1−F. The relation for P is

$$P_t = \left(1-\frac{1}{N}\right) P_{t-1} + \frac{1}{2N} P_{t-2}, \quad . \quad . \quad . \quad (1)$$

where the subscript refers to the time in generations since the establishment of the population. The explicit solution of (1) in terms of N and t is easily obtained, and was first given, to the best of our knowledge, by Malécot (1946). The exact formula is

$$P_t = \left(\frac{1-\frac{1}{N}-\sqrt{1-\frac{1}{N^2}}}{2\sqrt{1-\frac{1}{N^2}}}\right)\left(\frac{1-\frac{1}{N}+\sqrt{1-\frac{1}{N^2}}}{2}\right)^t - \left(\frac{1-\frac{1}{N}+3\sqrt{1-\frac{1}{N^2}}}{2\sqrt{1-\frac{1}{N^2}}}\right)\left(\frac{1-\frac{1}{N}-\sqrt{1-\frac{1}{N^2}}}{2}\right)^t \quad . \quad (2)$$

The exact value of P_0 and P_1 is 1. For t greater than 1, the simplified approximate formula

$$P_t = \left(1+\frac{1}{2N}\right)\left(1-\frac{1}{2N}\right)^t \quad . \quad . \quad . \quad (3)$$

differs from the exact value by a quantity of the order of $t/(16\,N^4)$,

which can almost always be neglected. (*E.g.* for N as small as 20 and t as large as 200, the error is of the order 1/12,800 ≅ ·0001). The even simpler form

$$P_t = e^{-\frac{t}{2N}} \quad . \quad . \quad . \quad . \quad . \quad (4)$$

has an absolute error of at most 1/2N and a relative error of approximately $(1/2N)-(t+1)/8N^2$. Table 2 gives the estimated values of t, the number of elapsed generations, the number of cultures with each such value, and the value of P_t calculated from (3) for the most probable N, 20, and for the upper bound of 40.

It may be worth while to make a few remarks here about the nature of this "inbreeding" in random mating finite populations, since many non-mathematical geneticists find it a confusing subject. Let us suppose a population segregating for a pair of alleles or karyotypes A and A′, and suppose that in the founding population (generation O) A has the frequency q_0 in both males and females. Then in the next generation ($t = 1$), the probability that an individual chosen at random is heterozygous is $2q_0(1-q_0)$. The probability that an individual chosen at random from the t-th generation will be a heterozygote will be $P_t \cdot 2q_0(1-q_0)$, and in this sense P_t measures the loss of heterozygosis due to inbreeding. However, in fact,[1] the loss of heterozygosis is entirely due to genetic drift from random sampling errors, and if q_t is the actual frequency of A in the t-th generation, then the probability that an individual chosen at random in the t-th generation will be a heterozygote is $2q_{t-1}(1-q_{t-1})$. In other words the Hardy-Weinberg equilibrium will be maintained in the population, so long as it is calculated from *current* gene frequencies, and any "inbreeding" is only relative to a particular reference point in the past. This is quite different from the real departure from the Hardy-Weinberg equilibrium in a population where close relatives mate more frequently than would be expected by chance.

Thus the probability that a random individual will be homozygous is $1-2P_tq_0(1-q_0)$. But what is the probability that a second individual chosen at random will be, say, A A, given that the first one was A A; what is the probability that two individuals chosen at random will be homozygotes of the same kind? The answer to these questions is not obvious. It is even less obvious what the probability is that all the individuals in the t-th generation are homozygotes of the same kind; that is that $q_t = 0$ or 1, although this probability must be large if P_t is close to zero. This probability can be obtained by the methods of Markoff chains, although the labour increases with N, or for large N by consideration of a diffusion process. Kimura (1954) has given the solution for large N in the form

$$H_t = 6q_0(1-q_0)e^{-t/2N} + 14q_0(1-q_0)[1-5q_0(1-q_0)]e^{-6t/2N} + R, \quad (5)$$

where H_t is the probability that both alleles are still present and R is of the order of magnitude of $e^{-15t/2N}$. For $6t/2N$ greater than 7

or t greater than 2·4N, even the third term is less than 0·001, and we have

$$H_t = 6q_0(1-q_0)e^{-t/2N} \quad . \quad . \quad . \quad . \quad (6)$$

This probability of no fixation is largest if $q_0 = 1/2$, as would be expected on intuitive grounds, so that even in this least favourable case for fixation, the probability that fixation has not occurred is only

$$H_t = \frac{3}{2} e^{-t/2N}, \quad . \quad . \quad . \quad . \quad (7)$$

or approximately 1·5 P_t.

TABLE 2

Estimated number of generations, t, *number of originally heterozygous cultures for each* t, *number of cultures no longer heterozygous, and values of the panmictic index* $P_t = 1-F_t$ *and of the maximum probability of remaining heterozygous for the most probable population size,* $N = 20$, *and the upper limit of population size,* $N = 40$

t	No. of cultures	No. not heterozygous	N = 20		N = 40	
			P_t	Prob. het.	P_t	Prob. het.
130	1	0	0·041	0·059	0·202	0·296
132	2	0	0·040	0·056	0·197	0·288
142	9	1	0·031	0·044	0·174	0·255
143	3	0	0·030	0·042	0·172	0·250
166	6	1	0·017	0·024	0·129	0·189
167	3	1	0·017	0·022	0·127	0 186
184	1	0	0·011	0·015	0·103	0·150
193	1	0	0·009	0·012	0·092	0·134
211	1	0	0·006	0·007	0·074	0·106
Total	27	3				

Table 2 gives the value of H_t, calculated from (7), for all cultures for N = 20 and N = 40. In table 2 the largest probability that both alleles are still present is for N = 40, t = 130 and is approximately 0·3. Even if we took this largest value as the actual probability for all the cultures, the probability that 24 out of the original 27 cultures with more than one chromosome present would not lose any chromosome is less than 10^{-7}. If we take account of the actual time values, and the fact that in one case all of *three* original chromosomes were preserved, this probability would become negligible. Evidently then, some agency was acting to maintain heterozygosis, and the most likely candidate is selection in favour of heterozygotes.

4. INBREEDING WHEN HETEROZYGOTES ARE FAVOURED

It is clear that when heterozygotes are favoured by selection, progress toward homozygosis under inbreeding will be greatly slowed. This process has been studied by the methods of Markoff chains by

Hayman (1953), Hayman and Mather (1953 and 1956), Reeve (1955), and Haldane (1956). Most of these authors considered regular systems of inbreeding, and the only connection with the present problem is that sib mating is equivalent to $N_m = N_f = 1$. Kimura (1955) has considered random mating in a finite population with genic selection, but not selection in favour of heterozygotes. The Markoff chain methods become excessively laborious for N as large as 20, involving 40×40 matrices, while Kimura's methods assume large N and might not be valid for $N = 20$, even if the formidable analytic difficulties were overcome. Accordingly recourse was had to Monte Carlo methods or in less elegant terms " bean bag genetics ", that is, to a sampling experiment. The model chosen gives the karyotypes A A, A A′, A′ A′, in the ratio $(1-s) : 1 : (1-s')$. Then if the gene frequency in the t-th generation is q_t, the expected gene frequency in the $(t+1)-th$ generation is

$$\tilde{q}_{t+1} = \frac{q_t - sq_t^2}{1 - sq^2 - s'(1-q)^2} \quad . \quad . \quad . \quad . \quad (8)$$

For a given value of s, the probability of fixation in a large number of generations is least for $s' = s$, since in this case equilibrium is at $q = 1/2$ and large or small values of q, with danger of fixation, are least likely. Accordingly s' was chosen to equal s, and (8) became

$$\tilde{q}_{t+1} = \frac{q_t - sq_t^2}{1 - s[q^2 + (1-q)^2]} \quad . \quad . \quad . \quad (9)$$

To minimise the chance of fixation, $\tilde{q}_0$ was then chosen to be $1/2$, and a sample of 2N was chosen from a binomial population with probability $1/2$. The resulting frequency of A was q_1. Then $\tilde{q}_2$ was calculated from (9) and a sample of 2N was chosen from a binomial population with the proportion $\tilde{q}_2$ of A's. The result of this sampling was the observed value of q_2, from which $\tilde{q}_3$ was calculated, and so on. The process was stopped after 170 generations (as representative of the actual cultures) unless fixation was reached before that time.

For $N = 40$, and $s = s' = 0{\cdot}1$, five trial populations reached fixation in 22, 29, 37, 45 and 55 " generations ". This result is significantly different from the observed 3 out of 27 using Fisher's exact test ($P = 0{\cdot}0002$). Evidently an advantage of 10 per cent. for the heterozygote is not enough to prevent fixation, even with the upper limit of 40 for the population size and with $q_0 = 1/2$, the value least favourable to fixation. Next the more probable population size of 20 was chosen, and $s = s'$ increased to $1/2$. Now two trials went 170 generations without reaching fixation. Finally two trials with $N = 20$ and $s = s' = 1/4$ gave no fixation in 170 generations, although a third reached fixation in only 14 generations. In general if q became small at some time t, but did not reach fixation, selection soon returned q to near $1/2$, so that the four artificial populations

that did not reach equilibrium in 170 generations ended with the rarer allele having a q of 0·35, 0·40, 0·42, and 0·45. Further trials with $s = 1/2$ and $s = 1/4$ were not made because of the labour and the fact that very many more trials would have had to be made before a significant difference from the observed proportion of 3 fixed out of 27 could be found. Thus it was not practicable to distinguish between $s = 1/2$ and $s = 1/4$ as the proper value, although $s = 0{\cdot}1$ was definitely too small. All that can safely be said is that for most of the chromosome pairs studied s and s' must have been greater than 0·1 and probably greater than 0·2. The three cultures that reached fixation may have been " unlucky " or may have had smaller values of s and s', or s and s' of opposite sign.

5. DISCUSSION

In *Drosophila pseudoobscura* and in *D. persimilis*, heterozygotes which carry two chromosomes of a pair with different gene arrangements but derived from the same geographic population are usually heterotic. However, Epling, Mitchell and Mattoni (1953, 1957) put forward what they believe to be an alternative hypothesis. It is well known that reduction of crossing over in a certain chromosome by a heterozygous inversion may be accompanied by increased recombination in some portions of the chromosomes in which crossing over is normally rare. Epling, Mitchell and Mattoni then postulate that under changing environmental conditions, the increased recombination in the residual genome of structural heterozygotes may aid in adapting to the changing conditions and consequently the offspring of structural heterozygotes will be favoured. It seems doubtful whether a second order effect such as this could actually give the heterozygotes as much advantage as they have in natural populations. As an explanation of the retention of the inversion heterozygotes under the fairly constant conditions of the laboratory cultures, the hypothesis of Epling *et al.* may safely be dismissed.

Nevertheless, heterosis is not an intrinsic property of inversion heterozygotes. Some inversions failed to display heterosis in experimental populations of *Drosophila pseudoobscura* kept at 16° C., or fed on nutrient media with certain yeasts (Dobzhansky and Spassky, 1954 and other work, see above). Yet it is exactly at that temperature that our laboratory strains have been kept and showed a persistence of the inversion polymorphism. This is not necessarily a contradiction ; relatively weak heterosis is hard to detect in experimental populations, and different geographic strains may have different temperature sensitivity ranges, just as the related species, *D. pseudoobscura* and *D. persimilis*, are known to have. It is even less necessary to suppose, as some writers have done, that the heterosis will lead to differential mortality of the inversion homozygotes and consequent disturbances of the ratios demanded by the binomial square rule. Such disturbances

have indeed been found in certain populations, both natural and experimental ones, but they are neither expected nor observed to be universal. In fact, an advantage of the heterozygote with respect to such factors as fertility and fecundity would not cause such disturbances.

Only a few studies have separated the individual components of adaptive value. Spiess, Ketchel and Kinne (1952) found the WT/KL inversion heterozygotes in *Drosophila persimilis* appreciably superior to the WT/WT and KL/KL homozygotes in fecundity. Spiess and Schuellein (1956) showed further that the WT/KL heterozygotes exceed the homozygotes in speed of development from egg to adult and in survival rates during the development. Finally, the heterozygotes possessed superior homeostatic buffering, expressed in lower environmental components of the variance estimates for the traits studied. Such superior homeostasis has also been found by Rosenbaum-Moos (1955) in *D. pseudoobscura* inversion heterozygotes. In this species, the over-all fitness of the ST/CH heterozygotes was found to be no higher than that of the ST/ST homozygotes, but much higher than that of the CH/CH homozygotes. However, the different components of fitness of these karyotypes are known to vary in their relative values quite appreciably in different environments (Heuts, 1947, 1948 ; Birch, 1955).

Inversion polymorphism is clearly a part of the adaptive machinery in natural populations of Drosophila. But no organic system is adaptive as such ; adaptedness is harmony between a living system and the conditions of its existence. The relative adaptive values of the heterozygous and homozygous karyotypes in Drosophila populations are exquisitely sensitive to variations in the environment ; and yet the populations are homeostatically buffered against environmental disturbances because they are composed of numerous genetic types with diverse environmental optima.

6. SUMMARY

Strains of *Drosophila pseudoobscura* and *Drosophila persimilis*, each descended from a single inseminated female collected in nature, have been kept in laboratory cultures for an estimated 130 to 211 generations (see table 1 for particulars). The strains were examined for inverted sections in their third chromosomes within a few generations after their capture. At that time, 26 strains contained two gene arrangements each, 12 strains one gene arrangement each, and 1 strain three gene arrangements. Re-examination of the same strains showed that only 3 of the 27 strains which were structurally polymorphic became uniform in the course of time.

Groups of flies estimated to consist of between 20 and 40 individuals were transferred to new food in each generation to maintain the strains. It can be calculated that, with this breeding system, the strains should have become homozygous with respect to most of the unfixed genes

and chromosomal structures which they contained originally. The failure of the 24 out of the 27 strains to become homozygous for the gene arrangements in third chromosomes shows that the inversion heterozygotes are superior in fitness to the inversion homozygotes.

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A SURVEY OF THE FREQUENCIES OF *BISTON BETULARIA* (L.) (LEP.) AND ITS MELANIC FORMS IN GREAT BRITAIN

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1. FOREWORD

Biston betularia, the Peppered Moth (previously placed in the genera *Amphidasys* and *Pachys*), is one of about 70 species of Macrolepidoptera occurring in this country which are at present in the process of changing their populations from light to dark. This is referred to as Industrial Melanism. In nearly every instance it results in the substitution of a complex pattern by a dark or black coloration. Industrial melanism is found, in fact, only among those species which depend for their survival by day on their efficiency in harmonising with the specialised background on which they rest, such as lichened trunks, boughs, rocks or wood. It is not found in those species which exhibit other types of protective coloration. The black mutant is nearly always inherited as a simple Mendelian dominant. In compiling a survey, such as the one about to be described, it was necessary to choose a species which had the following attributes :—it must be widespread, forming fairly continuous populations both in industrial and rural areas ; it must indulge in considerable periods of flight to encourage gene-flow ; and be easy to capture at mercury vapour traps or by other methods. *B. betularia* has all these qualifications. Accordingly, in 1952 I was fortunate enough to recruit a team of about 80 entomologists (which has since risen to over 150) living in different parts of the country, and to them I owe my sincere thanks for more than 20,000 records of this species which they have sent me in the last four years.

2. THE PHENOTYPES

Typical *B. betularia* is whitish overlaid with innumerable thin black lines and dots. At rest on lichened boughs it is often almost invisible. The first melanic appeared about 1848 in Manchester (Barrett, 1901), and is referable to as f. *carbonaria* Jordan (syn. *doubledayaria* Millière).

Another melanic form is known as *insularia* Th. Mieg. It is not as extreme as *carbonaria* and is variable. Occasionally it is difficult to differentiate from the *typica* form, and still less frequently from *carbonaria*. These two melanics are not allelomorphic (Kettlewell, 1953). Hitherto there has been no evidence of linkage. There is,

however, a distinct possibility that f. *insularia* may be controlled by a series of alleles. The three forms are figured in a previous paper (Kettlewell, 1956*a*). *Carbonaria* is unifactorial and dominant to the *typica* form, and *insularia*, which is also dominant or semi-dominant to the type, cannot be recognised phenotypically in the presence of *carbonaria*.

3. PREVIOUS INVESTIGATIONS

The following facts have been recorded by me and have a direct bearing on the distribution of the three phenotypes in Britain :—

(*a*) " To the human eye, *carbonaria* proved much better concealed on the lichen-free tree trunks, blackened by pollution near Birmingham, but this advantage was reversed in favour of the pale form in unpolluted country. *Insularia* possesses an intermediate advantage in both places " (Kettlewell, 1956*a*).

(*b*) Mark-release-recapture experiments showed that in the industrial area of Birmingham we recaptured more than twice as many f. *carbonaria* as *typica* in a total release of 584 of these two forms. In contrast, a release of 969 individuals into an unpolluted and heavily lichened wood in Dorset resulted in twice as many of the *typica* form being recaptured as of the f. *carbonaria*. In both experiments, the recaptures were usually made within 24 hours of the release.

(*c*) This was shown to be due to bird predation. Six species of birds were recorded as taking part in this and filmed as they captured resting moths whilst under observation (by Dr Niko Tinbergen). In Birmingham, Redstarts, *Phoenicurus phoenicurus* L., were seen to take 58 *betularia* which had been released in a 1 : 1 ratio, melanic : typical ; 43 were *typica* and 15 f. *carbonaria*. On the other hand, in the unpolluted wood of Dean End, Dorset, when both forms were released in equality, 5 species of birds were observed to eat 190 *betularia*, of which 164 were f. *carbonaria* and 26 f. *typica*. These findings provided evidence for stating that " the difference in cryptic coloration alone could be responsible for the rapid spread of the Industrial Melanics ".

(*d*) Nevertheless, we have provided certain basic evidence that there exist both physiological and behaviour differences between the melanic and typical forms (Kettlewell, 1956*b*).

4. EARLY HISTORY OF MELANIC SPREAD

Prior to the middle of the last century, there is every reason to believe that melanic forms were maintained in the population solely by recurrent mutation. For the next fifty years these melanics were much sought after by collectors, so that it is reasonable to suppose that their capture would be recorded assiduously when they first made their appearance. Table 1 gives a list of the earliest records

TABLE 1

TABLE 1

Earliest records of f. carbonaria *in Britain. Approximate selective advantages (S.A.) have been estimated for a period of 100 years on the assumption that the local frequency of f.* carbonaria *was not greater than 1 per cent. when first recorded*

County Locality (recorder)	1st *carbonaria* recorded	1900-06 survey (when known)	S.A.	Locality (recorder)	1952-56 frequency sample size	S.A.	Locality (recorder)	S.A. over total period
BERKSHIRE								
(Barrett) . . .	1885	...	...	...	11% (63)	...	Newbury (Saundby)	3%
CAMBRIDGESHIRE								
Cambridge (Farren) .	1892	" now seen every year "	...	(Farren)	93% (88)	...	Cambridge (H.B.D.K.)	14%
Ely (Cross) . . .	1895	...	...	...	...	...	...	15%
CHESHIRE								
Chester (Barrett) . .	Delamere 1860	83% sample 180	18%	Chester (Arkle)	94% (124)	5%	Chester (G. Smith)	10%
ESSEX								
Colchester (Harwood) .	1892	...	...	...	86% (822)	11%	Bradwell (Dewick)	11%
Dovercourt (Mathew) .	1902	...	...	...		14%	"	14%
GLOUCESTERSHIRE . .	...	" black not observed "	...	Stroud (Prideaux)	16% (185)	>6%	Nailsworth (Demuth)	>6%
HAMPSHIRE								
New Forest (Barrett) .	1897	...	...	...	10% (324)	4%	Eastleigh (Goater)	4%
IRELAND								
Castle Bellingham . . (Thornhill)	1894	...	...	...	7% (58)	3%	Belfast district (Wright)	3%
ISLE OF MAN								
(Cassall)	1904 2 sps.	...	...	...	13% (69)	6%	Santon (Hedges)	6%
KENT	...	" black not observed "	...	Farnborough (Christy)	73% (224)	>12%	E. Malling (Groves)	>12%
LANCASHIRE								
Manchester (Edleston) .	1848	" black prevalent. Type occurs "	...	Manchester (Clutton and Tait)	98% (350)	...	Manchester (Michaelis)	15%
LINCOLNSHIRE								
(Barrett) . . .	*c.* 1860	" both light and black "	...	(Fowler)	91% (158)	...	Louth (H.B.D.K.)	9%
LONDON DISTRICT								
Woodford (Mira and Bacot)	1897	37% (sample 27)	52%	Woodford (Main and Harrison)	90% (327)	8%	Whetstone (Lovell)	14%

S.A. = Selective advantage of f. *carbonaria* as calculated from Haldane's (1924) Table.

TABLE 1 (*continued*)

County Locality (recorder)	1st *carbonaria* recorded	1900-06 survey (when known)	S.A.	Locality (recorder)	1952-56 frequency sample size	S.A.	Locality (recorder)	S.A. over total period
MONMOUTH								
Newport (Wheeler) . .	...	" 1870 two forms about equal "	...	...	...	...	...	...
NORFOLK								
Kings Lynn (Atmore and Baker)	1892	" Prevalent " (=50%)	>60%	(Atmore and Baker)	77% (137)	<3%	Fritton (H.B.D.K.)	10%
STAFFORDSHIRE								
Cannock Chase (Barrett) .	1878	" all black " (= ≳80%)	>25%	(Frere)	90% (42)	3%	Cannock Chase (Richardson)	11%
SUFFOLK								
Ipswich (Morley and Pyett)	1894	...	...	...	75% (56)	10%	Lowestoft (Burton)	10%
SURREY								
Croydon (Gower) . .	1906	" black not observed "	...	Reigate (Meyrick)	77%(1615)	>13%	Woking (C. de Worms and Trundell)	>13%
					79% (1347)	>13%	Ottershaw (Bretherton)	>13%
SUSSEX	...	" black not observed "	...	(Hewitt)	16% (383)	>6%	Hastings (Astbury)	>6%
					26% (106)	>7%	Eastbourne (Ellison)	>7%
WARWICKSHIRE								
Birmingham . . .	...	" 50% black "	...	(Barrett)	87% (1611)	5%	Birmingham (H.B.D.K.)	5%
WESTMORLAND								
Kendal and Windermere (Moss)	1870	" black commoner than type " (= 50%)	14%	(Barrett)	49% (47)	...	Kendal (Birkett)	...
WILTSHIRE								
Marlborough (Prentice) .	1951	" black not observed "	...	Marlborough (Davis)	9% (460)	>45%	Marlborough College	>45%
YORKSHIRE								
Bradford (Butterfield) .	scarce 1876	" black now prevalent " (= >50%)	>10%	(Butterfield)	96% (508)	<10%	Bradford (Briggs)	10%
Huddersfield (Porritt) .	1861	" now only black " (= ≳80%)	≳15%	(Porritt and Morley)	99% (409)	...	Sheffield (H.B.D.K.)	17%

S.A. = Selective advantage of f. *carbonaria* as calculated from Haldane's (1924) Table.

of f. *carbonaria* from different localities, and it has been assumed that at these dates *carbonaria* would not be at a local frequency higher than say 1 per cent. In 1900, The Evolution Committee of the Royal Society began an investigation into the problem of industrial melanism. It is unfortunate that approximate percentages only are given (Doncaster, 1906), but by this date scientific opinion had evidently become conscious of the biological importance of the rapid spread of the melanic forms. Only on two occasions, in fact, are there actual figures for random samples. The same unfortunate shortcomings are found in the later investigations of Mera (1925) and Adkin (1925). In fact nowhere are the figures given which would have helped so much in the present investigation.

Certain information can, however, be deduced from these earlier records. It appears that f. *carbonaria* was taken for the first time in many widely separated places between 1848 and 1900. Whether this represents migration from one centre, or numerous discrete mutations, will be discussed later. Secondly, very shortly after the initial captures this melanic form increased rapidly in a brief space of time. Thirdly, until the end of the century, *carbonaria* was unknown in southern England. At this time it had a midland and eastern county distribution and was not taken in the London area till 1897. Fourthly, when these earliest records are considered with our recent data, the selective advantages (p. 64) for the *carbonaria* form can, with a reasonable degree of accuracy, be assessed for each locality over the period involved. The ability to do this in one of the most transient polymorphisms ever known, makes these earliest records most valuable.

5. PRESENT SURVEY

In view of the inadequate nature of the data on the frequencies of the melanic forms at any one period in the past, I decided in 1952 to obtain as much data on *betularia* as quickly as possible, with the object of providing figures for future reference. At the same time, a great deal of information has been collected, some of which is capable of being analysed at present.

Table 2 gives a list of frequencies of the three forms of *betularia* from many areas of Great Britain. Furthermore, the last column gives the estimated true frequency of *insularia* in the non-*carbonaria* fraction of the population (assuming no linkage), *carbonaria* being epistatic to this form. It will be appreciated that the higher the frequency of *carbonaria*, the greater the error in assessing the *insularia* frequency, and that even in large random samples from industrial areas the data are often inadequate.

TABLE 2

Phenotype frequencies of Biston betularia *and its two melanics, f.* carbonaria *and f.* insularia, *from 83 centres in Britain (1952-56)*

Locality	Observer	Per cent. T	Per cent. C	Per cent. I	Total	Estimated frequency per cent. I
BEDFORDSHIRE						
Woburn	S.H.K.	4·76	95·23	0	21	0
Leighton Buzzard	J.F.R.	15·43	82·28	2·29	175	12·90
BERKSHIRE						
Newbury	R.S.	79·37	11·11	9·52	63	10·71
CAMBRIDGESHIRE						
Cambridge	H.B.D.K. B.O.C.G.	4·54	92·95	3·41	88	42·85
CHESHIRE						
Chester	S.G.S.	5·65	93·55	0·81	124	12·50
Delamere	W.E.A.	6·68	90·86	2·46	569	26·92
Heswall	D.E.H.	6·82	93·18	0	44	0
Wirrall	A.C.	1·56	98·44	0	192	0
CUMBERLAND						
Penrith	W.F.D.	60·71	26·79	12·50	56	17·07
DERBYSHIRE						
Chesterfield	J.H.J.	0·59	99·41	0	170	0
DEVON and CORNWALL						
Torquay	F.H.L.	100	0	0	100	0
Ashburton	S.T.S.					
Bude	A.H.					
Plymouth	F.W.J.	100	0	0	500	0
Tavistock	D.J.W.					
Tiverton	F.H.L.					
DORSET						
Dean End Wood	H.B.D.K.	93·64	0·87	5·49	346	5·54
Broadmayne	V.W.P.	97·02	0	2·98	168	2·98
ESSEX						
Bradwell on Sea	A.J.D.	6·69	86·25	6·57	818	49·54
Westcliffe on Sea	H.C.H.	5·95	85·55	8·5	353	58·82
GLOUCESTERSHIRE						
Hardwicke	R.D.	38·89	23·61	37·5	72	49·09
Nailsworth	A.R.	48·65	15·68	35·67	185	42·31
HAMPSHIRE						
Bournemouth	F.M.B.C.	60·00	30·00	10·00	40	14·29
Chandler's Ford	B.G.	82·1	10·49	7·41	324	8·27
Borden	D.W.	65·43	20·29	14·28	3095	17·92
Fordingbridge	P.J.B.	86·84	5·26	7·89	38	8·33
Winchester	R.S.M.W.	84·85	15·15	...	33	0
Fleet	A.W.R.	47·31	46·03	6·66	315	12·35
HEREFORDSHIRE						
Malvern	R.K.J.	46·30	27·80	25·90	54	35·90
HERTFORDSHIRE						
Bishops Stortford	C.C.	4·76	89·18	6·06	231	56·01
Rothamsted	C.B.W.	11·08	83·69	5·23	325	32·08
Tring	L.G.	16·89	74·30	9·81	214	38·18

TABLE 2 (*continued*)

Locality	Observer	Per cent. T	Per cent. C	Per cent. I	Total	Estimated frequency per cent. I
IRELAND						
Belfast district	W.S.W.	93·10	6·90	...	58	0
Dublin district	E.S.A.B.	96·30	3·70	...	27	0
ISLE OF MAN						
Santon	A.H.	50·70	13·11	36·20	69	41·67
ISLE OF WIGHT						
Freshwater	K.E.B.	...	...	...	50	...
KENT						
Bromley	M.G.M.	9·26	88·89	1·85	54	16·66
Folkestone	A.M.M.	46·43	42·86	10·71	140	19·75
East Malling	D.E.	...	...	...	...	...
Maidstone	J.R.G.	21·43	73·21	5·36	224	20·00
LANCASHIRE						
Formby	N.G.L.	5·32	94·68	...	94	0
Southport	K.L.G.	10·00	90·00	...	120	0
Manchester		0	98·00	2·00	350	100
LEICESTERSHIRE						
Market Harborough	H.A.B.	11·83	84·95	3·32	93	21·43
LINCOLNSHIRE						
Grimsby	G.A.T.J.	7·41	88·89	3·75	81	33·35
Louth	H.B.D.K.	6·33	91·13	2·53	158	28·57
LONDON AREA						
Whetstone	R.L.	5·81	90·21	3·98	327	40·63
MIDDLESEX						
Pinner	W.E.M.	9·14	84·95	5·91	558	39·28
NORFOLK						
Cromer	J.B.	20·93	67·44	11·63	43	35·71
Fritton	H.B.D.K.	14·60	77·37	8·03	137	35·48
NORTHANTS						
Wellingborough	P.J.G.	14·24	79·88	5·90	288	29·31
OXFORDSHIRE						
Oxford district	H.B.D.K. P.M.S. G.V.	41·00	34·03	24·97	717	37·84
Steeple Barton	H.B.D.K.	49·13	34·26	16·61	289	25·26
SCOTLAND						
Glasgow	H.B.D.K.	10·33	89·66	...	29	...
Kinloch Rannoch	R.L.	100	...	...	285	...
Newtonmore	G.H.	100	...	...	100	...
SOMERSET						
Portishead	J.A.B.	50·00	20·00	30·00	10	37·50
SUFFOLK						
Beccles	E.T.G.					
Lowestoft	J.B.	17·86	75·00	7·14	56	28·57

TABLE 2 (*continued*)

Locality	Observer	Per cent. T	Per cent. C	Per cent. I	Total	Estimated frequency per cent. I
SURREY						
Cobham	J.B.P.	17·98	76·86	5·17	484	22·32
Cranleigh	H.B.D.K.	50·00	39·30	10·70	28	17·65
Ottershaw	R.F.B.	13·73	79·93	6·34	1435	31·60
Woking	C.deW. E.T.	16·84	76·66	6·88	1615	25·88
SUSSEX						
Brighton	R.W.D.	41·18	52·94	5·88	17	12·50
Eastbourne	R.E.E.	40·57	26·42	33·02	106	44·87
East Grinstead	M.G.	66·70	53·30	...	21	...
Hastings	C.F.A.	74·15	15·67	10·18	383	12·07
Petworth	P.D.	79·66	11·86	8·47	59	9·61
NORTH WALES						
Dolgelley	T.T.	95·37	2·78	1·85	108	1·90
Llandudno	J.A.T.	100	...	...	100	0
HEREFORD						
Ross on Wye	J.E.K.	39·29	21·43	39·29	28	50
WARWICKSHIRE						
Birmingham	H.B.D.K.	8·94	87·09	3·97	1611	30·77
Cannock Chase	R.P.D. A.R.	7·5	90·00	2·50	40	25·00
Tysoe	T.T.	22·22	66·67	11·11	18	33·33
Wyre Forest	H.B.D.K.	31·34	64·18	4·48	67	12·50
WESTMORLAND						
Kendal	N.L.B.	40·43	48·94	10·64	47	20·83
WILTSHIRE						
Warminster	R.J.	83·33	9·26	7·41	54	8·17
Marlborough Col.	C.R.P. R.W.C.V. S.L.S.	79·78	8·91	11·30	460	12·41
YORKSHIRE						
Aysgarth	H.B.D.K.	4·00	94·00	2·00	100	33·33
Bradford	J.B.	1·97	95·67	2·36	508	54·54
Grassington	G.deC.F.	...	100	...	60	...
Sheffield	H.B.D.K.	0·5	99·00	0·50	409	50·00

Throughout this paper the letters T, C, I stand for *Biston betularia* f. *typica*, f. *carbonaria* and f. *insularia* respectively.

A distribution map (fig. 1) of the frequencies of the three forms shows :

(*a*) A correlation between the frequency of the melanic forms and the industrial areas of Britain.

(*b*) A high frequency of *carbonaria* throughout eastern England from north to south, though far removed from industrial centres. This is, in my opinion, the indirect effect of long continued smoke fall-out carried by the prevailing south-westerly winds from central England.

(*c*) Western Britain, with the exception of Cheshire, Lancashire and Westmorland (Kendal) is virtually melanic free.

(*d*) Northern Scotland has no melanic *betularia* but in the Glasgow district f. *carbonaria* is about 90 per cent.

(*e*) In Ireland, *carbonaria* has been recorded, first in 1894 from the Belfast district and aso Dublin, but is of rare occurrence. It has been found nowhere else.

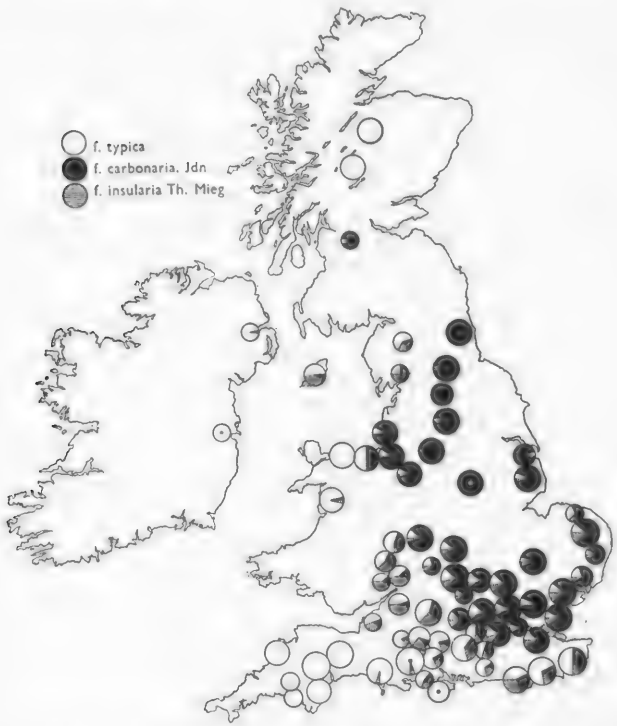


FIG. 1.—A frequency map of *Biston betularia* and its two melanics, f. *carbonaria* and f. *insularia* (1952-56), comprising more than 20,000 records from 83 centres in Britain.

(*f*) The map shows multiple clines running out from the higher *carbonaria* frequencies in the centre of England. There may be a rapid decline from east to west as, for instance, from Cheshire to North Wales, or from Birmingham to Wyre Forest, or they may be gradual as always found in an easterly direction.

(*g*) The highest frequency of f. *insularia* phenotype is, for the most part, found in the Gloucester and Severn Valley district of England.

6. ANALYSIS OF F. *CARBONARIA* FREQUENCIES

(a) *Evidence of mutation rate*

If one reviews the first county records, it is indeed difficult to resist the conclusion that the new mutant has radiated outwards from an original centre of mutation near Manchester. There is no doubt that following the original capture in 1848, the nearest counties to Lancashire were the next to record its appearance ; Cheshire in 1860, Yorkshire in 1861, Staffordshire in 1878, and Westmorland in 1870. The London district, on the other hand, and southern England did not report a *carbonaria* until about twenty years later [1897). It is significant also that the eastern counties of Norfolk, Suffolk and Cambridge all recorded their first *carbonaria* practically simultaneously between 1892 and 1895.

All this is consistent with the spread of a successful gene with no ecological barriers, from its centre of origin. Furthermore, on the Continent, the same spread was taking place, being recorded by A. Hofmann from Hanover in 1884, the Netherlands and Thuringia in 1888 " and in the next few years in various parts of the Rhine Valley, indeed he [Hofmann] thinks that its progress was up the Rhine " (Barrett, 1901).

In a short series of evening releases undertaken in open country near Louth, Lincs., in 1956 I attempted to find the distance which *betularia* flies per night. Using different releasing points, different markings, and several collecting centres of known distance apart, I was able to show that of 93 releases, 6 travelled 1½ miles, and 9 over half a mile within 48 hours. At Fritton in Suffolk, of 78 releases over three occasions, 16 were recaptured three-quarters of a mile distant, 12 within 24 hours. From this, having regard to the random flight of the releases, it can be accepted that *betularia* frequently flies a mile per night, probably much farther. Nevertheless, dispersal cannot be accepted as the actual cause of the widespread distribution of f. *carbonaria*. There is considerable evidence that recurrent mutation, at a fairly high rate, also takes place. *Carbonaria* has constantly appeared in isolated centres separated from others by usually impassable barriers. Thus it was taken in Dublin about 1950, near Belfast in 1894, the Isle of Man in 1904, and in 1956 at Torquay, each locality being separated by fifty miles or more from the nearest possible contacts. It is, in fact, likely that the *carbonaria* allele has a high mutation rate. This is in contrast with certain melanics of other species, such as f. *nigra* of *Ectropis consonaria*. In this country, only on two occasions has the melanic mutation successfully maintained itself, first near Maidstone in Kent, and secondly near Stroud, Gloucestershire, where a sample of 121 recorded by me in 1956 were 43 per cent. of the melanic form. It would appear that this melanic mutation has not yet taken place in an industrial centre in this country. It has, however, in Germany where it has become an industrial melanic.

(b) Period of adjustment

Haldane (1924) has pointed out that if in Manchester in 1848 the *betularia* population was 99 per cent. the *typica* form, and if by 1898 it was 99 per cent. f. *carbonaria*, this represents an approximate 30 per cent. advantage of the black form over the light. Magnitudes, only rather less than this, are borne out by table 1.

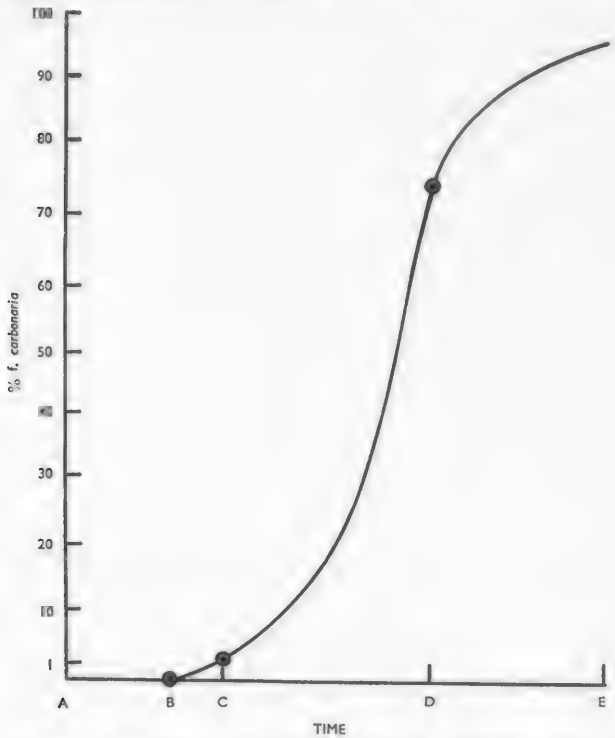


FIG. 2.—Diagram showing rate of increase of a melanic (dominant) mutant with a mutation rate of one in a million, assuming a constant advantage of the heterozygote throughout (which in practice will not occur), with a 30 per cent. selective advantage over its typical form.

It is now necessary for us to consider, therefore, the time lag between the original mutation and its arrival at the 1 per cent. level. At frequencies below this, it is unlikely to have been recognised in the population because of the poorer techniques in sampling used at that date. Let us examine an hypothetical case and assume that in a local population of the order of a million, a single melanic mutation has occurred. With a 30 per cent. advantage, it would take about 29 years to reach the 0·1 per cent. level, and 38 years to 1 per cent. Fig. 2 is a diagram showing the type of spread expected from a new

mutant from the time of its origin until it has reached a state of balanced polymorphism or, alternatively, has eliminated its allele from the population. Present data are in agreement with this type of curve. The period under present consideration is represented by BC on the abscissa, and if, in fact, there was only one mutation in Manchester, and by 1848 *carbonaria* has reached 1 per cent. of the population, we can say that this original mutation took place in about 1810, which is approximately fifty years after the commencement of the Industrial Revolution.

During this initial period (BC), the black mutants, though having great cryptic advantage, nevertheless would find themselves in a gene-complex entirely fitted for the specialised pattern of the *typica* form. It is of interest that many of the earliest examples of f. *carbonaria* which I have obtained by searching innumerable old collections, are different from the majority of heterozygous *carbonaria* found to-day. Plate I shows two rows of probable heterozygous *carbonaria*, the right-hand one of which is comprised of insects, all of which were caught in the last century. They bear no resemblance to f. *insularia*. In contrast, the left-hand column shows five modern heterozygotes, and it will be noted that they differ considerably from those collected seventy years previously. Many of the earliest specimens show white markings on all the wings in both sexes greatly in excess of what are generally found to-day. The present-day heterozygote only occasionally shows minor degrees of white markings on the wings. On the other hand, the majority of *carbonaria* have white dots around the head and at the base of the wings. In the Sheffield area and, to a lesser degree, in other centres (Chester for example), these spots are also disappearing in present-day specimens, leaving a completely black insect. This must be brought about by modifying genes through the effect of natural selection. It provides evidence that during this period the gene-complex was adjusting itself to attain its greatest advantage. Furthermore, it suggests that in the early days f. *carbonaria* had not full dominance. We are at present attempting to verify this by out-crossing *carbonaria* into West Country stock whose complex has had no experience of this mutant.

Of even more interest is the situation I have recently found in regard to physiological advantages and disadvantages. It is accepted among lepidopterists that nowadays an excess of melanics occur in backcross broods. Table 3*b* gives a list of my own results of backcross *betularia* broods fed on unwashed Oxford Sallow (*Salix* sp.) which corroborates this fact. It will be seen that there is a significant excess of melanics. In contrast to this, Doncaster (1906) records figures which deviate in the opposite direction for backcross broods occurring between 1900 and 1906 (table 3*a*). It is probable that these also were fed on unwashed foliage. Both sets of data are homogeneous but are significantly different. (The $\chi^2_{(1)}$ is 13·27.) It appears, therefore, that in its early phases, the melanic mutant was not at a physiological

advantage to the *typica*, and that only after a considerable period of adjustment did it become so. It is possible then that certain major modifying genes, previously adapted for keeping the complicated typical pattern in check, freed from cryptic responsibilities, were now

TABLE 3

A comparison between early and recent backcross broods of Biston betularia *segregating for* typica *and* carbonaria *showing a deficiency of the two forms in opposite directions*

(*a*) Earliest broods recorded 1900-1905				(*b*) Recent broods 1953-1956				
T	C	Total	Breeder	T	C	Total	Breeder	Brood no.
123	109	232	Bacot	14	22	36	H.B.D.K.	B/2/52
57	47	104	Main and Harrison	7	10	17	H.B.D.K.	19/53
18	11	29	Fletcher	1	5	6	H.B.D.K.	30/53
57	50	107	Harrison	28	30	58	H.B.D.K.	15/54
				1	2	3	H.B.D.K.	19/54
				14	39	53	H.B.D.K.	11/54
255	217	472	C = 46·6%	65	108	173	C = 62·4%	

(*c*) 2×2 Table			
T	C	Total	Year
255	217	472	1900-1906
65	108	173	1953-1956
320	325	645	

(*a*) $\chi^2_{(1)} = 2{\cdot}90$; P 0·089

(*b*) $\chi^2_{(1)} = 10{\cdot}20$; P 0·0014

(*c*) $\chi^2_{(1)} = 13{\cdot}27$; P 0·0003

able to exploit themselves in bringing about the best physiological advantage. This period then (BC) must be regarded as a time during which the gene-complex was adjusting itself to the new mutant. The disappearance of a complicated pattern may make it easier for alleles, previously adjusted to this end, to contribute more in other directions.

(c) *Period of rapid spread*

In theory, when once adjusted, the new mutant is free to spread, provided the selective pressure is maintained. As in the earlier periods, because backcross matings will leave a higher percentage of melanic offspring, this increase will proceed with great rapidity up to a time when 50 per cent. of the population is of the *carbonaria* form, and thereafter it will tend to slow down at a speed directly proportionate

to the increasing *carbonaria* frequency when the number of heterozygotes in the population will commence to drop as pointed out by Fisher (1937). If we review the progress of *carbonaria* frequencies in those few localities (table 4) where it stands between 1 and 40 per cent. for the short time they have been under observation, there are, indeed, indications of rapid spread. In each, it will be seen that the *carbonaria* frequency has risen significantly. It is, however, possible that the very high selective advantage, apparent in the Marlborough figures, may be due to the initial sample being inadequate. This period of rapid spread is shown on the graph (fig. 2) as CD during which, under certain conditions, it is probable that f. *carbonaria* can change from being 10 per cent. of the local population to 70 per cent. within a period of ten to fifteen years, which reflects accordingly a minimum selective advantage of from 35 to 23 per cent.

(d) *Period of slow elimination of f. typica or alternatively a balanced polymorphism*

The data in table 2 show that in every industrial area f. *carbonaria* is now at least 85 per cent., but in no large sample is the value 100 per cent. Even after one hundred years, Manchester and Sheffield still have 1-2 per cent. of non-*carbonaria* forms, and Lincolnshire 9 per cent. Furthermore, the only actual sample figures available are for Chester, which show that f. *carbonaria* rose in the last fifty years from 83 to 94 per cent. only, giving a selective advantage of 5 per cent., after having achieved the earlier frequency from 1 per cent. in forty-five years (= selective advantage 18 per cent.). All the available evidence goes to show that f. *typica* continues in the population however great are the apparent selective pressures against it. This period is referred to as DE in the diagram (fig. 2), and may represent many hundreds of years, depending on unknown variables. It is probable that by now, after experiencing one hundred generations, the new mutant will have succeeded in achieving a gene-complex suitable for its optimum expression, and that by this time the heterozygote will be at an advantage to both the homozygotes. This will have been brought about by Natural Selection. Modifying genes, disadvantageous in the homozygous state, but advantageous as heterozygotes, will have become more closely linked to the new mutant and one would probably find a figure below the expected 3 : 1 ratio as the result of heterozygous pairings in the wild, because of the elimination of the homozygous *carbonaria*. This will inevitably be followed by a balanced polymorphism.

As a corollary of this, it can be anticipated that the rare f. *typica*, being eliminated on nearly every occasion because of its conspicuousness, and now finding itself in a gene-complex no longer adapted to it but to the melanic, should, in theory, diverge from its previous fixed pattern. I have, therefore, recently commenced to collect all

the f. *typica* from districts where it occurs at a very low frequency. With the small numbers at present available, and the difficulty in analysing the degree of divergence, all that can be stated is that considerable variation does occur. Whether this is more than is found in samples from districts where f. *carbonaria* is absent, I am so far unable to tell.

Conclusions on carbonaria *frequencies*

The autocatalytic or sigmoid curve, suggested by Haldane (1924) as representing the likely course of spread of f. *carbonaria*, has been fully borne out. The present investigation shows selective advantages for this mutant of a slightly lower magnitude than that suggested by him, and this, no doubt, reflects more accurate data for present-day frequencies than were available to him. Alternatively, an apparent lower selective advantage would be brought about if a stable polymorphism was taking place in populations with a high frequency of f. *carbonaria*.

7. ANALYSIS OF F. *INSULARIA* FREQUENCIES

The early history of f. *insularia* is unsatisfactory in every respect. It is generally agreed that it appeared about the same time as f. *carbonaria*, but in certain districts, particularly in the south, before it. Doncaster (1906) tried to differentiate " two distinct forms ", a light and a dark, and he may in fact be correct. Furthermore, the earlier breeders were confused on finding that backcross *carbonaria* × *typica* broods, on some occasions, " produced intermediates " (= *insularia*), whilst on others Mendelian segregation took place (Doncaster, 1906).

Insularia occurred in Ireland in 1894, in Scotland (Kincardine) some time prior to 1906, and was found at the end of the last century in most central industrial areas. It was recorded on the Continent (Belgium) in 1886. All observers agree that it is an industrial melanic, and that it gets rarer following the upsurge of *carbonaria*.

It is not surprising that f. *insularia*, being intermediate in appearance between f. *typica* and f. *carbonaria*, is found most frequently at the present time on the periphery of industrial areas, outside centres with a high frequency of *carbonaria*, in whose presence it is impossible to detect phenotypically. Background scoring efficiency (Kettlewell, 1955), for the few releases undertaken by me, give *insularia* a position intermediate between f. *typica* and f. *carbonaria* in both industrial and rural environments. It is, in fact, admirably suited for resting on boughs covered with Pleurococcus and not lichens. Nevertheless, it must be pointed out that, in contrast with *carbonaria*, the phenotype frequency of *insularia* has never been found higher than 40 per cent.

Insularia has the great drawback, previously referred to, that it varies from those individuals which are indistinguishable from f. *typica* to those which are as dark as f. *carbonaria*. The frequency figures, unlike those for f. *carbonaria*, are, therefore, subject to personal error.

To estimate this variability, four observers, all living within three miles of each other, and each rated as a most reliable lepidopterist, have supplied independent data over the last few years. It is clear from the table that the estimates of *carbonaria* percentages do not differ very markedly. Comparing the four values by use of a 2×4 contingency table does, in fact, disclose some heterogeneity, since $\chi^2_{(3)} = 7{\cdot}88$, and the 5 per cent. point is $7{\cdot}81$. Although this is formally significant, it does not contradict the view that variations between observers are probably small in respect of *carbonaria* classifications, since the total sample size of 3534 is large enough to detect very small differences.

Percentage phenotypes				
T	C	I	Total	Name	I. estimated frequency
18 per cent.	77 per cent.	5 per cent.	484	J.B.P.	22·32
14 ,,	80 ,,	6 ,,	1435	R.F.B.	31·60
18 ,,	78 ,,	4 ,,	1191	C.deW.	18·80
15 ,,	74 ,,	11 ,,	424	E.T.	40·54

To make a similar test for *insularia*, we have examined the 2×4 contingency table based on the *insularia* and *typica* figures. (This is because the *carbonaria* class is largely uninfluenced by the possibility of misclassification, as explained above.) We find $\chi^2_{(3)} = 23{\cdot}64$, for which $P < 0{\cdot}001$. Thus a very appreciable degree of heterogeneity is revealed in spite of the much smaller sample (777) now used, and this is reflected proportionately in the estimated frequencies of the *typica* and *insularia* forms which for this district vary, with comparable methods of collecting, between 18 and 40 per cent.

Nevertheless, in spite of identification difficulties, f. *insularia* offers many points of considerable interest. As pointed out by Haldane (personal communication), a triangular graph of the three phenotypes (fig. 3) shows that high values of f. *insularia* are associated with *carbonaria* frequencies of from 10 to 30 per cent. Districts showing this, for the most part, are centred around the Severn Watershed and Gloucestershire which, without doubt, receive constant pollution fall-out from Bristol and South Wales. Also the Isle of Man, which is subject to occasional but definite smoke drift from the industrial areas of Lancashire, when the wind is in an easterly direction, has a high *insularia* frequency of 36 per cent. In those areas where *carbonaria* is common, the frequency of visible *insularia* falls, and the random sample, therefore, becomes less accurate, varying in direct proportion to an increased *carbonaria* frequency.

Unfortunately earlier records of f. *insularia* are, for the most part, lost, and what I have been able to extract is largely circumstantial.

For example, the Giles Collection of Lepidoptera in the Folkestone Museum was amassed between the years 1880 and 1890. It was a local collection and in it is a series of 18 *betularia*, 12 *typica* and 6 *insularia*. They are by appearance not bred, in fact many of both forms are worn, and it is likely that this is a small random sample, and reflects correctly a frequency of about 33 per cent. *insularia* at this date. *Carbonaria* did not appear in Folkestone until a very much later date, about 1927 (Morley, personal communication). As will be seen from table 2, the present *insularia* frequency is about 11 per cent., with *carbonaria* standing at 42 per cent., which gives an estimated

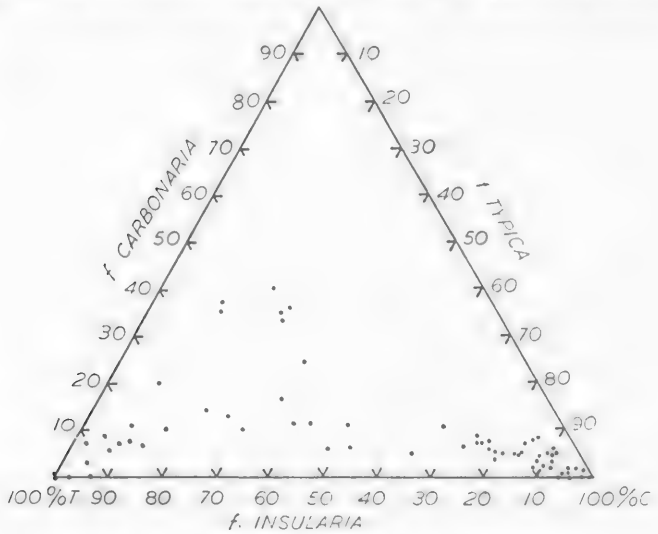


FIG. 3.—Graph showing frequencies of the three phenotypes of *Biston betularia* in Britain.

insularia frequency, on the assumption of there being no linkage, of 17 per cent. It has, in fact, dropped 16 per cent. in thirty years, during which time the frequency of *carbonaria* has risen from approximately 1 to 42 per cent. The true frequency of f. *typica* would, therefore, have increased during this period from 66 to 83 per cent. If this is true, f. *carbonaria* has expanded at the expense of f. *insularia* and not f. *typica* and it suggests that *insularia*, in the presence of heterozygous *carbonaria*, may interact in a disadvantageous manner.

At the upper end of the *carbonaria* frequency, there appear to be only one set of early figures available. Arkle (1901) took a sample of 180 *betularia* at electric light in Chester, and this can be compared with a sample of 124 taken during 1956 by S. Gordon Smith. In a period of over fifty years, the estimated frequency of *insularia* has dropped by only 7·5 per cent. in the population in spite of its cryptic

disadvantage. Furthermore, if one includes the combined figures for Cheshire, the *insularia* frequency is still 22·76 per cent.

Percentage phenotypes					
Year	T	C	I	Total	I Estimated frequency
1901	13 per cent.	83 per cent.	3 per cent.	180	20 per cent.
1956	6 ,,	93 ,,	0·81 ,,	124	12·5 ,,

The *insularia* records obtained in the last few years are a good deal more helpful. Nowhere have we so far obtained a phenotype frequency at a level higher than 40 per cent. This can be interpreted in any one of several ways. Either on every occasion f. *carbonaria* has swept

TABLE 4

Frequency of f. carbonaria *recorded in the last four years for districts having a low* carbonaria *frequency*

Locality (observer)	Year	Per cent. T	Per cent. C	Per cent. I	Total	Estimated *insularia* frequency
Oxford . . (P. Sheppard)	1952	45·65	28·26	26·09	46	36·37
	1954	42·50	34·20	23·50	219	35·45
	1956	34·82	39·70	25·40	224	42·22
Wiltshire . . (Marlborough College)	1951	76·67	1·66	21·67	60	22·03
	1952					
	1955	86·30	7·19	6·48	139	6·98
	1956	76·38	11·81	11·81	254	13·39
Glos, Nailsworth. (A. Richardson)	1952	43·14	11·76	45·10	51	51·11
	1956	50·75	17·16	32·09	134	38·74
Hants, Bordon . (D. Wright) .	1952	59·66	21·33	19·01	647	24·16
	1953	64·44	19·53	16·03	599	19·92
	1954	69·39	18·38	12·23	941	14·90
	1955	66·08	22·02	11·90	908	15·25

This gives the following selective advantages for f. *carbonaria*: Oxford 12·2 per cent., Wiltshire 44·6 per cent., Gloucestershire 12 per cent., Hampshire 2 per cent. (approx.).

through the population, an increase in pollution having taken place, thereby favouring this more extreme mutant and enabling it to overtake *insularia*; or, alternatively, homozygous *insularia* are at a considerable disadvantage to the heterozygotes. A third explanation could be that *insularia* has partial linkage to *carbonaria*, so that fewer *insularia* phenotypes appear in the population when the *carbonaria* mutant becomes common. Table 4 does, in fact, suggest, with the

exception of Oxford, that *carbonaria* increases at the expense of *insularia* and not of f. *typica*.

If we assume that there is no linkage, we are able to graph the estimated frequency of *insularia* in any population, in which it may be to a great extent concealed by *carbonaria*. The higher the *carbonaria* frequency, however, the greater the error because of the paucity of the *insularia* sampled. Fig. 4 shows the result of graphing all estimated frequencies shown in table 2 in which the total sampled was larger than fifty individuals. The data suggest the following deductions :—

(1) When f. *carbonaria* and f. *insularia* simultaneously enter a population consisting of f. *typica* only, f. *insularia* will rise to about

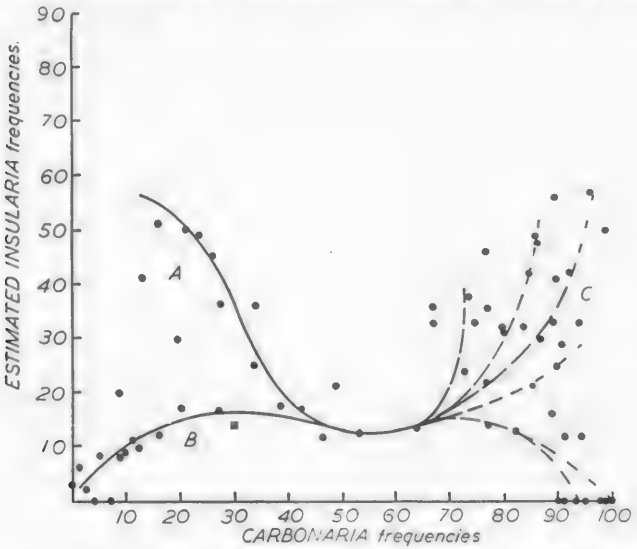


FIG. 4.—Graph showing frequencies of *insularia* for the majority of districts in Britain (plotted against *carbonaria* frequencies).

15 per cent., but no higher as long as the *carbonaria* frequency remains under 10 per cent. (District B).

(2) When f. *carbonaria* enters a population which has a high frequency of f. *insularia* (40 per cent.), it is likely that this will drop to this same level of 15 per cent. (District A).

(3) *Carbonaria* frequencies of between 40 and 65 per cent. are associated with the lowest gene frequencies of *insularia*, and this coincides with the most rapid spread of f. *carbonaria* (fig. 2).

(4) Thereafter, a rising *carbonaria* frequency is associated with *insularia* frequencies varying from high to low (District C). It is of interest then to find that each of the three main groupings, designated with a letter, correspond geographically with the different areas of Britain in the majority of recordings. Thus group A, with a high

frequency of *insularia* is largely constituted by the Severn Valley, Gloucestershire and Oxfordshire ; group B the counties of Berkshire, Wiltshire, Hampshire and Dorset ; while group C represents the whole of the eastern half of England from Yorkshire to London, an area which corresponds with the earlier map of *carbonaria* as drawn up by Adkin (1925).

For the purpose of analysis, it is convenient to divide the graph (fig. 4) into two portions : below 50 per cent. *carbonaria* frequency and above.

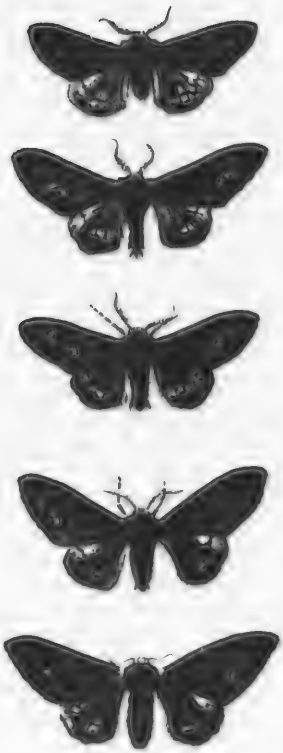
Below a 50 per cent. *carbonaria* frequency, it is evident that no matter at what level the *insularia* has been maintained in the population, the entry of *carbonaria* into it will canalise the *insularia* for a period of time at an approximate 15 per cent. level. It is possible on the other hand that this may represent an equilibrium frequency in which f. *insularia*, in the presence of prolonged but slight pollution fall-out, is capable of adjusting itself in the population at this level in a state of balanced polymorphism. It is a more likely explanation, however, that it is in a transient state, in which *carbonaria*, the more extreme melanic, is replacing a less suitable one on the presence of ever-increasing air pollution. It could, however, be a further indication that *insularia*, in the presence of *heterozygous carbonaria*, is at a disadvantage.

Above a 50 per cent. *carbonaria* frequency (fig. 4), the estimated *insularia* values vary from 0 to 65 per cent., the majority being high except in populations practically entirely overrun by f. *carbonaria*, where the *insularia* samples are always inadequate. This can be brought about by one of two alternative ways. The initial cryptic advantage of *insularia*, being now largely lost in the *carbonaria* phenotype, it is likely that heterozygous *insularia* have some definite physiological advantage in the presence of *homozygous carbonaria*. Alternatively, it could be argued that over levels of 65 per cent. *carbonaria* frequencies, the *insularia* cryptic advantage is largely lost, and *insularia* would find itself floating in a *carbonaria* population, and subject to drift. This could constitute a new type of circumstance, allowing drift, but in my opinion it is most unlikely that *insularia* has a completely neutral physiological value apart from its initial cryptic advantage. Furthermore, if homozygous *insularia* are eliminated as seems possible, drift cannot be considered as an explanation.

Standard statistical analysis shows a highly significant degree of heterogeneity ($\chi^2_{(38)} = 99{\cdot}86$) in the proportions of f. *insularia* in populations with over 65 per cent. *carbonaria*, and it is evident that f. *insularia* continues to maintain itself within a *carbonaria* population even after losing its cryptic advantages and, in fact, may even expand. On the other hand, slow elimination is indicated by the subsequent history of the only recorded early frequency known to me, which indicates that in Chester the estimated frequency of *insularia* has dropped 7·5 per cent. in 55 years, but the sample for this assumption is inadequate. It must be remembered that at the beginning of this

MODERN HETEROZYGOTES

OF CARBONARIA



ANCIENT CARBONARIA

1. Coll. F. Bond, about 1872
2. Coll. Gregson, old pin
3. Coll. Gregson, old pin
4. Coll. Sidney Webb
5. Coll. Cooke, old pin

Plate

Ancient and modern heterozygotes of *Biston betularia* f. *carbonaria*. The former were extracted from old collections made in the last century and have many white markings. Specimens like these are but rarely found to-day.

(Photograph by John Haywood from painting by Christine Court, Oxford).

period 3 per cent. of the population were still appearing as f. *insularia* which would, on most occasions, be eliminated because of their cryptic disadvantage in heavily polluted districts. Only future records will be able to settle what really happens, and their foundation is now laid in this paper. At the same time, it has served to point out that the natural history of the T-C-I complex in this rapidly changing countryside is likely to raise problems of unusual interest.

8. SUMMARY

1. The early history of *Biston betularia* and its two melanic forms in Britain, *carbonaria* and *insularia*, has been recorded. Selective advantages have been estimated for the period of over 100 years since the commencement of industrial melanism, and shown to be similar to those suggested by Haldane (1924) on the ground of theory, but slightly smaller.

2. The present survey includes over 20,000 records of *betularia* from many districts of Britain which have been made by over 150 observers. A table of these is given, and a frequency map has been prepared from them. The data show correlation between the industrial centres and high frequency of f. *carbonaria*, the more extreme melanic. *Carbonaria* is also abundant throughout the eastern half of England ; this is probably the result of the indirect effects of smoke-drift following the prevailing south-westerly wind. The south-western parts of England and the whole of northern Scotland are free of melanics.

3. The rate of spread of *carbonaria* follows a sigmoid curve. The original light form is maintained at a very low frequency in all industrial populations. This may eventually lead to a balanced polymorphism if the heterozygous *carbonaria* are at an advantage to either homozygote.

4. The highest phenotype frequencies of *insularia* are found in populations having 10 to 30 per cent. *carbonaria*, and these are for the most part centred around the Severn Watershed, Gloucestershire and Oxfordshire. *Insularia* phenotype never occurs higher than 40 per cent., and this may be because the homozygote is at a disadvantage.

5. A graph, showing the estimated frequencies of *insularia*, has been prepared. It has been shown that *carbonaria*, when between 20 and 50 per cent. of the population, expands at the expense of *insularia* but not of f. *typica*.

6. In populations of more than 65 per cent. *carbonaria*, the *insularia* gene frequency may be either high or low, in spite of ever increasing loss of its earlier cryptic advantages.

7. This paper is intended to be a foundation for future records which may disclose the true behaviour of two dominant genes both rapidly and simultaneously spreading through a population.

Acknowledgments.—I wish to thank all the observers without whose help this paper would not have been possible. I am grateful to the Nuffield Foundation who have enabled me to undertake this work. I am indebted to Professor J. B. S. Haldane, F.R.S., for helpful suggestions and for the privilege of his analysis of this paper. Also to Dr P. M. Sheppard who has not only contributed records of his own, but has given me much advice. I have greatly benefited from the co-operation of Dr E. B. Ford, F.R.S., also Dr A. J. Cain and Mr M. H. Williamson. I would like to thank Miss Christine Court for her help in the preparation of maps and graphs, and Mr John Haywood for his photography.

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A FURTHER CASE OF DOMINANCE INTERACTION IN *MUS MUSCULUS*

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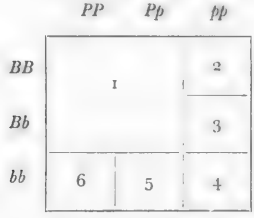
It was reported by Snell (1931) that in the presence of homozygous non-agouti (*a*) and pink-eyed dilution (*p*), the heterozygous condition black-brown (*Bb*) is distinguishable, as a lighter colour, from the homozygous black (*BB*). Wallace (1953) pointed out that this constitutes a reduction of the dominance of *B* by the *p* locus, and demonstrated that the converse is also true, namely that the *b* locus reduces the dominance of *P*. Thus the nine genotypes available at these two loci comprise six distinct phenotypes (table 1).

TABLE 1

Interaction of b *and* p *in* aa DD *genotypes (from Wallace, 1953)*



In a stock involving these same factors, in which *PP* was backcrossed to *Pp*, and in which Maltese dilution (*d*) was also segregating, Wallace has also noticed two colour phenotypes in *Bb dd* and *bb dd*, namely dark and pale black dilutes, and dark and pale brown dilutes. On her suggestion that the "darks" in each case are *PP* and the "pales" *Pp*, I have tested for the presence of *p*, several "darks" and "pales" among both the black and the brown dilutes from the backcross. In fourteen cases out of seventeen, the *p* genotype conformed to expectation. Since for each animal tested there was an equal chance of a right and a wrong classification, a simple χ^2 testing the equality of the number of right and wrong diagnoses gives the probability of obtaining this result by chance. χ^2 is 7·1176 for 1 d.f., probability $<0{\cdot}01$. Thus it may safely be concluded that, in the presence of *dd*, further genotypes at the *b* and *p* loci are distinct. These interactions may be summarised as follows (table 2).

The remaining phenotypic distinctions possible in *B* and *P* are being investigated in this Department, and it is hoped that a full table of interactions in *dd* genotypes may be published shortly as a

counterpart to that for *DD* genotypes. It should perhaps be pointed out that in all cases non-agouti mice are being used. It is possible to make many of the distinctions on agouti mice, if the non-yellow part of the fur is examined. Thus Snell's insistence on *aa* is not absolutely necessary, but it is helpful.

In order to confirm the present result and to examine the possible effect of minor colour factors, the genotypes 1 to 4 of table 2 were each reproduced from matings not segregating at the *p* locus, *i.e.* from matings whose progeny could be classified with complete certainty. These were :

(i) *PP Bb aa dd* × *pp bb aa dd*
(ii) *PP Bb aa dd* × *PP bb aa dd.*

Five mice of each genotype were skinned at approximately 28 days old, making twenty skins in all. These were displayed, each set of

TABLE 2

Interaction of b *and* p *in* aa dd *genotypes*

	PP	*Pp*
Bb	1	2
bb	4	3

five separately, and the skins in each set were numbered 1 to 5. Fifteen people in the Department who were not associated with the experiment were asked independently to say which were the pale and which the dark sets of skins. Among both the blacks and the browns, their classification, in all cases, coincided with the *Pp* and *PP* classification. Thus the effect of the *P*/*p* gene substitution is indisputable.

Each person was then asked to arrange the skins within each set in order of increasing intensity. From the tabulation of these orders, it was found that in none of the sets was there unanimous agreement upon any order, but in all sets one or two of the skins were classified by the majority as definitely paler, or darker, than the rest. Thus it is clear that slight variation exists within each phenotype : this may be due to the segregation of minor colour factors or to environmental differences such as age and its effect on the stage of moult. The median skin, that is, the one occurring most frequently as number 3 in the tabulation of orders, was selected to demonstrate the contrasts between the four phenotypes and can be seen in this Department.

It is conceivable that minor genetic and environmental fluctuations could, in a replication of this investigation, produce slightly different degrees of contrast between the four phenotypes—and in fact some overlapping may be possible. Short of complete standardisation of

residual genotype and environment, a laborious task, the exact interrelations cannot be established. However, such a replication, if it confirmed the contrasts so far observed, would enable the less clear distinctions such as may exist between the *BB* and *Bb* genotypes and the *PP* and *Pp* ones, to be made with confidence. For this reason, the fuller investigation now being carried out, includes a replication of the present work.

To summarise : Wallace's work demonstrates the power of homozygous *b* to reduce the dominance of *P* over *p*. The present work shows that, with the help of a third factor diluting the wild black colour, namely *d*, the effect of *b* is even stronger, this dominance being incomplete in heterozygous as well as homozygous *b* genotypes. There is some variation within each phenotype, which may be due to minor genetic factors or to environmental causes.

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A BALANCED FOUR-POINT LINKAGE EXPERIMENT FOR LINKAGE GROUP XIII OF THE HOUSE MOUSE

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1. INTRODUCTION

LINKAGE group XIII in the house mouse was established by Fisher in 1950 when he reported recombination between polydactyly (*py*) and leaden (*ln*).

Polydactylous mice have appeared in numerous stocks for some years. As an inherited entity, polydactyly has been reported by Murray (1932), Strong (1934), Fortuyn (1939), and finally by Holt (1945). It was, however, of very poor manifestation, and was not usable as a genetic factor until Holt (1945) had selected for good manifestation after outcrossing. Leaden, a recessive factor, arose in the same inbred chocolate-brown stock in which Murray found polydactylous mice (Murray, 1933).

The next factor, now known to belong to this linkage group, that occurred, was splotch (*Sp*), which is a dominant and was reported by Russell (1947). In 1949 Strong and Hollander reported looptail (*Lp*), which is a badly manifesting dominant of poor viability, and is therefore difficult to work with. At this stage, Fisher reported his *py-ln* linkage, and this was quickly followed by Dickie and Woolley (1950) who reported a new recessive factor fuzzy (*fz*) linked to *ln* with a recombination value of $40{\cdot}9\pm3{\cdot}7$ for female heterozygous parents, and $41{\cdot}5\pm4{\cdot}5$ for males. They also reported a linkage between *fz* and *Sp* of $41{\cdot}3\pm5{\cdot}2$.

Fisher set out the position for the *py-ln* linkage fully in 1953. He obtained a recombination value of $40{\cdot}3\pm1{\cdot}77$ for females, and $23{\cdot}0\pm2{\cdot}00$ for males, which is a striking sex difference. Data for the linkage of *Lp*, *ln*, *Sp*, and *fz*, appeared in 1954 (Snell, Dickie *et al.*) :

TABLE 1

Linkage of Lp, ln, Sp, fz

	Lp	*ln*	*Sp*	*fz*
Heterozygous ♂	35·1	5·8	31·6	
,,	38·1	8·5	39·6	

These data show a sex difference but not of the magnitude found by Fisher.

From these preliminary tests, it was evident that the markers in this linkage group served to map a greater map distance than is

available in any other linkage group in the house mouse. An accurate knowledge of recombination and interference along this chromosome would help to verify or modify recent theories of genetical recombination developed mainly by Owen. For this reason, Fisher set up matings designed to map this chromosome as accurately as possible using the four markers *py*, *ln*, *Sp*, and *fz*. The factor *py* is thought to be situated very close to *Lp*, and in the stocks in this Department *py* is the easier factor to work with. In this paper four-point data for the long segment *py-ln-Sp-fz* will be analysed and the results discussed. They are derived from the material built up by Fisher over the last five years, and have not yet been reported.

2. THE FACTORS INVOLVED

A brief description of the factors *py*, *ln*, *Sp*, and *fz*, will be given here with particular reference to any difficulties encountered while working with them.

2.1. *Polydactyly*

The polydactylous mice in this Department originated in one of Fisher's inbred lines, the TS line, at the Galton Laboratory in January 1942, from which a polydactyl line was set up. Prior to this occurrence, polydactyl mice appeared in a light-headed (*te te*) line, but test matings were shortlived and no polydactyl mice were obtained (Holt, 1945).

Polydactylous mice are readily observable at birth. With very few exceptions, polydactyly has been confined to the back feet, affecting either or both feet. When one foot is affected, it is more commonly the right foot (Holt, 1945). This was confirmed by Engel (1956).

The condition is very variable in expression, and all stages have been recorded from a slight abnormality where the first toe appears shorter and thicker than normal, to a severe form where there are seven complete toes. When both feet are affected, one foot is often affected differently from the other.

Holt (1945) has given the early history of polydactyly in considerable detail and showed that it is a recessive incompletely penetrant factor of highly variable expression. Inbreeding and outcrossing to several lines which had never shown polydactyly improved its manifestation considerably. Further selection by Fisher (1953) established within a year a stock in which it is invariably manifest. It appears that suppressors of unequal intensity inhibit *py* manifestation, one of which is in the Vth chromosome probably between agouti and pallid (Fisher, 1950). By selection, these suppressors have been removed.

In the linkage experiment to be analysed, homozygous polydactyly is manifest mainly as bilateral polydactylism. Of the animals affected on one foot, approximately two-thirds will be shown to be homozygous and one-third heterozygous. It is conceivable that further selection could make the factor still less recessive. This would be in agreement with Holt's (1945) suggestion that *py* may have been a dominant initially, but by natural selection acting through modifiers, the heterozygote has tended to resemble the advantageous homozygote as suggested by Fisher (1928).

2.2. *Leaden*

Leaden, first reported by Murray (1933), is a completely recessive factor with 100 per cent. manifestation in the homozygous state. It is a dilution factor, and the homozygotes do not appear to be less viable than the heterozygotes. Classification can be made at about six days.

2.3. *Splotch*

Splotch, *Sp*, is a dominant factor manifest by white spotting on the belly and occasionally on the back. The feet have white socks, and the tail may be partially white. It interacts with other spotting factors, *e.g.* piebald (*s*) and dominant pied (*W*) which can make classification difficult in some stocks. Classification can be made at about eight days.

2.4. *Fuzzy*

Fuzzy is a completely recessive factor with perfect manifestation. The hair on fuzzy mice is thinner and faintly wavy and the vibrissæ also are wavy. Fuzzy mice appear to be weaker in the litter initially but eventually grow to normal size. This is verified by a deficiency of homozygotes in the records.

2.5. *Conclusions*

This survey of the markers in linkage group XIII reveals some difficulties. Leaden is an excellent factor to work with, having perfect viability and manifestation. Fuzzy mice are of lowered viability in the litter. Splotch viability is normal but there is a slight danger of misclassification. Polydactyly has manifestation troubles but the *py* manifestation was relatively constant in the period when the data to be analysed in this experiment were collected.

3. DESIGN OF EXPERIMENT

There are eight possible quadruple heterozygotes for four linked markers, and eight possible modes of gamete formation. The eight heterozygotes were made up and backcrossed to a triply recessive stock of *py fz ln* mice of 100 per cent. *py* manifestation. The data from such a series of eight crosses can be arranged as an 8×8 Latin square in which the columns represent the eight modes of gamete formation, and the rows the eight quadruply heterozygous genotypes (table 2). If we let the three segments between the four markers *fz-Sp-ln-py* be (1), (2), and (3), respectively, then the eight modes of gamete formation can be designated by (0), (1), (2), (3), (12), (23), and (123), such that (0) represents no recombination, (1) represents recombination in segment (1), and so on.

The letters A-H correspond to the eight pairs of complementary genotypes

A	*Sp*	*fz ln py*
B	*fz Sp*	*ln py*
C	*Sp ln py*	*fz*
D	*Sp py*	*fz ln*
E	*fz Sp ln py*	+
F	*fz Sp py*	*ln*
G	*Sp ln*	*fz py*
H	*fz Sp ln*	*py*

The eight possible heterozygotes were made up and some effort was made to collect approximately equal data from each heterozygote

(table 3) so as to ensure balance. Balance is achieved when there are data in coupling and repulsion for each possible pair of factors. In a four-point test, sets of only four heterozygotes which are balanced can be selected. In this experiment, however, there are data for the eight possible heterozygotes so ensuring balance.

TABLE 2

8×8 Latin square

Parental genotypes	Modes of gamete formation							
	(0)	(1)	(2)	(3)	(12)	(13)	(23)	(123)
1. $\frac{fz\ +\ ln\ py}{+\ Sp\ +\ +}$	A	B	C	D	E	F	G	H
2. $\frac{+\ +\ ln\ py}{fz\ Sp\ +\ +}$	B	A	E	F	C	D	H	G
3. $\frac{+\ Sp\ ln\ py}{fz\ +\ +\ +}$	C	E	A	G	B	H	D	F
4. $\frac{fz\ +\ ln\ +}{+\ Sp\ +\ py}$	D	F	G	A	H	B	C	E
5. $\frac{fz\ Sp\ ln\ py}{+\ +\ +\ +}$	E	C	B	H	A	G	F	D
6. $\frac{fz\ Sp\ +\ py}{+\ +\ ln\ +}$	F	D	H	B	G	A	E	C
7. $\frac{+\ Sp\ ln\ +}{fz\ +\ +\ py}$	G	H	D	C	F	E	A	B
8. $\frac{fz\ Sp\ ln\ +}{+\ +\ +\ py}$	H	G	F	E	D	C	B	A

TABLE 3
Observed data

4. ANALYSIS OF THE DATA

The data for the female and male heterozygotes are summarised in table 3. The modes of gamete formation are shown along the top of the table, and the eight parental genotypes which can be seen from table 2 are shown along the side. Each pair of complementary genotypes A-H is split into its components, with the totals shown, these corresponding to the letters of the 8×8 Latin square. In the table the top figure of a complementary pair represents *Sp* mice and hence from table 2 their exact genotypes can be found. For each mode of gamete formation there are two columns, the first giving the female data, and the second the male data.

Before proceeding to the actual analysis, some comments will be made on the selection of data which are compiled in the 8×8 Latin squares.

The greatest difficulty encountered in these data was the variable manifestation of *py*. In the early stages, when the manifestation of

TABLE 3

Observed data

Parental genotypes (see table 2)		Modes of gamete formation																	
		(0)		(1)		(2)		(3)		(12)		(13)		(23)		(123)			
		♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂
1.	Sp	134	63	91	34	13	4	64	23	5	2	40	3	4	...	1	1		
	+	104	53	96	32	3	4	83	29	4	2	58	8	3	2	...	...		
		238	116	187	66	16	8	147	52	9	4	98	11	7	2	1	1	703	260
2.	Sp	49	102	36	65	2	4	26	30	...	...	23	19	1	...	...	1		
	+	52	101	30	57	5	9	45	50	1	1	12	16	3	3	1	...		
		101	203	66	122	7	13	71	80	1	1	35	35	4	3	1	1	286	458
3.	Sp	109	111	54	52	10	7	62	54	3	2	51	6	4	...	3	1		
	+	98	96	60	70	9	7	54	34	1	...	38	19	1	1	1	1		
		207	207	114	122	19	14	116	88	4	2	89	25	5	1	4	2	558	461
4.	Sp	30	84	26	51	2	11	11	49	...	3	11	12	1	...	...	...		
	+	23	105	21	64	2	5	29	27	1	2	14	8	...	1	1	1		
		53	189	47	115	4	16	40	76	1	5	25	20	1	1	1	1	172	423
5.	Sp	27	60	27	33	2	2	22	20	...	...	13	7	...	...	1	...		
	+	38	86	18	40	3	11	18	29	...	2	13	7	1	...	2	...		
		65	146	45	73	5	13	40	49	...	2	26	14	1	...	3	...	185	297
6.	Sp	27	38	19	19	1	8	22	14	...	1	16	7	1	1	...	...		
	+	30	57	26	26	5	4	24	21	1	...	18	9	1	...	...	1		
		57	95	45	45	6	12	46	35	1	1	34	16	2	1	...	1	191	206
7.	Sp	96	54	52	34	5	5	56	25	2	...	32	4	2	...	...	...		
	+	57	45	62	35	10	2	50	37	2	...	49	11	3	...	3	...		
		153	99	114	69	15	7	106	62	4	...	81	15	5	...	3	...	481	252
8.	Sp	43	34	34	38	7	...	27	35	2	...	22	7	1	...	...	...		
	+	43	40	27	28	7	6	29	22	1	...	16	5	2	...	...	1		
		86	74	61	66	14	6	56	57	3	...	38	12	3	...	...	1	261	216
		960	1129	679	67[illegible]	86	89	622	4[illegible]9	23	15	426	148	28	8	13	7	2[illegible]37	2573

F

py was being built up, or when a new factor was introduced to the stock, e.g. *fz* and *Sp*, the *py* manifestation was greatly upset and varied considerably between matings. The main problem in the data presented here is, however, the identity of animals polydactylous on one foot. Animals polydactylous on both feet were almost invariably

TABLE 4

Distribution of polydactylous mice

	Female	Male
Polydactylous on both feet . .	1371	1193
Polydactylous on neither foot .	1466	1380
Polydactylous on one foot . .	345	374
	3182	2947

py py, and those normal on both feet *py* +, but breeding tests showed that animals polydactylous on one foot could be either *py py* or *py* +.

In the discussion on viability it will be shown that animals manifesting *py* are no less viable than those not manifesting *py*. If it is assumed that any disturbance in the *py py* : + *py* ratio, which is expected to be 1 : 1 in a backcross linkage experiment, is due to manifestation some estimate of the proportions of animals polydactylous on one foot which are homozygous and heterozygous can be made. In table 4,

TABLE 5

Distribution of mice polydactylous on one foot

	Female	Male
py py	63·77 per cent.	75·00 per cent.
+ *py*	36·23 ,,	25·00 ,,

the data are split into three classes—(i) non-polydactylous, (ii) polydactylous on one hind-foot, and (iii) polydactylous on both hind feet. Data from female quadruple heterozygotes are separated from those from male heterozygotes.

The animals which show polydactyly on one foot are distributed among the other two classes such that the ratio *py py* : + *py* is 1 : 1. It is seen (table 5) that approximately two-thirds of this doubtful class is *py py*.

When this doubtful class was disregarded, the segregations in many matings became much more normal. It was therefore thought best, for the purposes of analysis, to disregard this class entirely. Before doing this, however, the distribution of this doubtful class amongst the eight modes of gamete formation was ascertained, and found to

be incompatible with the remainder of the data. Equally noticeable were the numerous animals appearing in the rare cross-over classes.

The other difficulty in some of the data was *Sp* classification, but selection of data for good *Sp* manifestation should remove any source of error. For most of the data, however, this difficulty did not arise, as selection and familiarity with the gene virtually eliminated any possible misclassification. As in the case of animals polydactylous on one foot, misclassification of *Sp* was often obvious by virtue of excessive *Sp* animals in the rare cross-over classes.

4.1. *Preliminary analysis*

Various preliminary tests are necessary before recombination can be safely estimated by the simple addition of the column totals in the 8×8 Latin squares. These tests may be classified into two types. Firstly, various tests may be done to test whether the data are heterogeneous. If so, the reliability of any estimates of recombination will be doubtful. Secondly, there are tests to detect viability and manifestation effects. If only one gene is of lowered viability, recombination can be estimated by adding the column totals. In this case, each cell of the 8×8 square will be affected equally by the viability effect. However, if two or more genes are of lowered viability, there will be genotypic viability differences and recombination must be estimated using a computation to remove such differences. The procedure for such computations has been developed by Fisher and set out by Fisher (1949) and Parsons (1957*a*) for three-point data. The former is a good first approximation, and the latter yields a solution to any desired degree of accuracy by an iterative process.

As a preliminary test of heterogeneity, a general χ^2 test which tests the agreement of observations and proportionate expectations based upon column and row totals may be calculated. For this test, columns (2), (12), (23), and (123) are neglected as the entries in these columns are very small. This leaves a 4×8 table. For the female data χ^2 was 16·58 for 21 degrees of freedom such that $0{\cdot}7<P<0{\cdot}8$ and for the male data $\chi^2_{21} = 30{\cdot}36$ ($0{\cdot}05<P<0{\cdot}1$). Neither of these tests reveal significant heterogeneity. Heterogeneity can occur as a result of poor data, or variable recombination according to the parental heterozygote, or finally as a result of differential genotypic viabilities. It may be concluded that such effects, if present, are probably negligible.

It is to be expected that the members of a pair of complementary genotypes will be unequal because of viability and manifestation disturbance. However, if the data are reliable, such deviations from the expected 1 : 1 ratio for no disturbance within a complementary pair should not be heterogeneous amongst the eight possible parental quadruple heterozygotes. Furthermore, there should be no differences in the magnitude of the disturbance encountered according to the sex of the quadruply heterozygous parent.

TABLE 6*a*

Heterogeneity tests for members of complementary pairs

Genotypes	Data arranged for testing (0)	(1)	(3)	(13)	♂	♀	Total	Total including (2), (12), (23), (123)
A *Sp*	197	101	60	23	184	197	381	400
fz ln fy	157	87	56	27	146	181	327	349
Totals	354	188	116	50	330	378	708	749
B *Sp fz*	151	125	36	23	162	173	335	345
ln fy	153	128	45	22	162	186	348	368
Totals	304	253	81	45	324	359	683	713
C *Sp fy ln*	220	60	81	29	176	214	390	408
fz	194	58	87	21	178	182	360	371
Totals	414	118	168	50	354	396	750	779
D *Sp fy*	114	38	87	42	145	136	281	298
fz ln	128	52	102	38	176	144	320	337
Totals	242	90	189	80	321	280	601	635
E *fz Sp ln fy*	87	106	62	36	151	140	291	306
+	124	130	51	60	189	176	365	388
Totals	211	236	113	96	340	316	656	694
F *fz Sp fy*	65	77	56	43	122	119	241	254
ln	87	85	95	66	179	154	333	351
Totals	152	162	151	109	301	273	574	605
G *Sp ln*	150	72	116	20	153	205	358	377
fz fy	102	55	88	20	114	151	265	279
Totals	252	127	204	40	267	356	623	656
H *Sp fz ln*	77	86	42	57	94	168	262	277
fy	83	97	47	57	123	161	284	302
Totals	160	183	89	114	217	329	546	579

The procedure for carrying out such tests can be illustrated by considering the first complementary pair in table 6, namely *Sp* and *fz ln fy*. The observed number of *Sp* mice is 400 and of *fz ln fy* 349. If there is no disturbance, equality would be expected. Assuming a 1 : 1 ratio, a χ^2_1 testing the agreement of observed and expected

was 3·47 ($0·05<P<0·1$) (table 6*b*), which is suggestive of some disturbance.

In the heterogeneity test to find out whether the *Sp* : *fz ln py* ratio varies in the data, columns (2), (12), (23) and (123) of the 8×8 Latin squares are neglected, as the data in these columns are often inadequate to give an accurate χ^2 test. For the four remaining columns a χ^2_3 test may be carried out to test the agreement of observed and expected frequencies calculated on the basis of row and column totals. This test gave $\chi^2_3 = 1·91$ ($0·5<P<0·7$) which is not significant.

Finally, it is of interest to find out whether each sex behaves similarly in regard to these deviations. The total mice in each sex is given for each genotype in a 2×2 table (table 6*a*). Proportionality

TABLE 6*b*

χ^2 tests from data in table 6*a*						
Genotypes	χ^2_6 for sex differences	P	χ^2_1 for over-all viability	P	χ^2_3 for heterogeneity between members of a complementary pair	P
A	0·80	$0·3<P<0·5$	3·47	$0·05<P<0·1$	1·91	$0·5<P<0·7$
B	0·16	$0·5<P<0·7$	0·74	$0·3<P<0·5$	0·82	$0·8<P<0·9$
C	1·23	$0·2<P<0·3$	1·76	$0·1<P<0·2$	1·96	$0·5<P<0·7$
D	0·56	$0·3<P<0·5$	2·40	$0·1<P<0·2$	1·86	$0·5<P<0·7$
E	0·003	$P>0·9$	9·69	$P<0·01$	7·75	$0·05<P<0·1$
F	0·43	$0·5<P<0·7$	15·55	$P<0·001$	3·86	$0·2<P<0·3$
G	0·0001	$P>0·9$	14·64	$P<0·001$	1·41	$0·7<P<0·8$
H	2·84	$0·05<P<0·1$	1·08	$0·2<P<0·3$	0·28	$P>0·9$

of rows and columns is tested with a contingency χ^2_1 applying Yates' correction for continuity. For complementary pair A, $\chi^2_1 = 0·80$ ($0·3<P<0·5$) which is clearly not significant.

These tests are applied to the remaining seven pairs of complementary genotypes in table 6. In some cases there are very serious deviations from the 1 : 1 ratio expected from members of complementary pairs, but such deviations are maintained throughout the data as is shown by the non-significance of the heterogeneity tests involved. No significant sex difference is detected for any of the complementary pairs.

The conclusions that may be drawn from this test are (i) there are disturbances affecting the expected 1 : 1 ratio of members of complementary pairs, (ii) such disturbances are maintained throughout any complementary pair in which they may exist, and (iii) there is no detectable difference in behaviour between the sexes.

The result of this analysis brings us to the question of the nature of such disturbances, and more important, the question of their effect, if any, on the recombination values to be estimated. A preliminary and crude test may be done by examining single factor ratios obtained

directly by addition from the observed progeny (table 7). Equality of these ratios is tested with a simple χ^2_1.

TABLE 7

Single factor ratios

Factor	*fz*	*Sp*	*ln*	*py*
Normals	2892	2745	2637	2846
Mutants	2518	2665	2773	2564
Total	5410	5410	5410	5410
χ^2_1	25·86	1·18	3·42	14·70
P	P<0·001	0·2<P<0·3	0·05<P<0·1	P<0·001

From these totals it is obvious that there is, as expected, a deficiency of *fz* and *py* mice.

The problem of polydactyly is more complex. It is known that there is a deficiency of polydactylous mice, by virtue of the discard of the doubtful animals which were polydactylous on one hind foot only. This, however, does not eliminate the possibility of a viability effect, although from the appearance of young polydactylous mice, this is unlikely. Polydactylous mice can be classified at birth and in this Department they are never left more than three days before initial classification. Full classification for all genes cannot be completed until the mice are eight to ten days old. Viability effects of polydactyly which may have occurred between initial recording and full classification, can be tested by noting the number of *py* mice and normal mice dying in that period out of a given total. For 2596 mice classified in such a manner, the following table is obtained.

TABLE 8

Viability of polydactyly

	Classified at birth	Died before full classification	Total
Polydactylous	1174	43	1217
Non-polydactylous	1327	52	1379
Total	2501	95	2596

The proportionality of rows can be tested with a χ^2 applying Yates' correction for continuity. For this table $\chi^2_1 = 0{\cdot}05$ ($0{\cdot}8<P<0{\cdot}9$). Thus there is no detectable difference between the mortality of polydactylous and normal mice between initial recording

and full classification. The deficiency of polydactylous mice compared with normals can therefore be assumed to be mainly an effect of poor manifestation.

An additional test can be done to test any possible interactions between the four factors. If we ignore *Sp* and *ln*, and consider *fz* and *py*, the eight pairs of complementary genotypes can be split into four pairs consisting of a *fz py* : + + contrast, and a *fz* + : + *py* contrast :

Genotype	+ +	*fz py*	Total	Genotype	*fz*	*py*	Total
A	400	349	749	B	345	368	713
E	388	306	694	C	371	408	779
F	351	254	605	D	337	298	635
G	377	279	656	H	277	302	579
Total	1516	1188	2704		1330	1376	2706

The ratio + + : *fz py* is significantly different from the expected 1 : 1 ratio for no disturbance ($\chi_1^2 = 14{\cdot}64$, $P<0{\cdot}001$). However, a heterogeneity test on the four components of this contrast to ascertain whether the deviation is consistent throughout the data is not significant ($\chi_3^2 = 3{\cdot}62$, $0{\cdot}3<P<0{\cdot}5$). In the case of the *fz* + : + *py* contrast, the over-all ratio is not significantly different from unity ($\chi_1^2 = 0{\cdot}78$, $0{\cdot}3<P<0{\cdot}5$). The heterogeneity test on the four components of this contrast is also non-significant ($\chi_3^2 = 5{\cdot}19$, $0{\cdot}1<P<0{\cdot}2$). If the factors *Sp* or *ln* were affected by viability, or if there were any interactions, the above heterogeneity tests might have been significant.

This somewhat lengthy series of preliminary tests gives a lead to the method of computation to be used in the estimation of the recombination fractions. It has been shown that *fz* is the only factor which is of reduced viability when homozygous, and that there are no interactions with the other factors. This viability effect is therefore equally distributed in each cell of the 8×8 tables, and in the estimation of recombination by simple addition of the column totals, the effect will cancel out.

Partial manifestation of polydactyly will bias the results if the effect is not estimated and removed. A relatively simple computation deals with this difficulty, and will be given in the next section.

In conclusion, these preliminary tests have shown that (i) the results are not heterogeneous, (ii) there is a simple viability effect due to *fz*, and (iii) there is a manifestation effect of *py*. Hence, the use of a relatively simple computation to remove bias due to poor *py* manifestation will provide accurate estimates of recombination without recourse to the difficult and lengthy computations of the type given by Fisher (1949) for the case where there is a significant genotypic differential viability effect.

4.2. *Estimation of recombination*

The mode of computation to be used in a balanced linkage experiment when one of the factors is imperfectly penetrant has been set out by Parsons (1957*b*) for three-point data and extended briefly to four-point data.

Let the frequencies of the eight modes of gamete formation (0), (1), (2), (3), (12), (13), (23) and (123) be p, q, r, s, t, u, v and w, respectively such that

$$p+q+r+s+t+u+v+w = 1$$

and let λ = misclassification of *py*.

Admitting misclassification of *py*, eight linear equations can be obtained from the 8×8 Latin square which will easily yield the eight modes of gamete formation :

$$\begin{aligned} p+\lambda(s-p) &= \mathrm{P} \\ q+\lambda(u-q) &= \mathrm{Q} \\ r+\lambda(v-r) &= \mathrm{R} \\ s+\lambda(p-s) &= \mathrm{S} \\ t+\lambda(w-t) &= \mathrm{T} \\ u+\lambda(q-u) &= \mathrm{U} \\ v+\lambda(r-v) &= \mathrm{V} \\ w+\lambda(t-w) &= \mathrm{W} \end{aligned}$$

In these equations P, Q, R, S, T, U, V, and W are the observed column totals of the 8×8 Latin square. Taking the above equations in the pairs (P, S), (Q, U), (R, V), and (T, W), the eight modes of gamete formation may easily be estimated.

The misclassification λ may be calculated using the method given by Parsons (1957*b*) for three-point data.

Thus we sum the proportion of *py* in each of eight possible pairs of genotypes, one member of each pair being polydactylous and the other identical to the first but non-polydactylous. Hence it is required to find the sum of :

$$\frac{\text{total observed } fz\ Sp\ ln\ py \text{ mice}}{\text{total observed } (fz\ Sp\ ln\ py + fz\ Sp\ ln) \text{ mice}} + \ldots \text{ to 8 terms}$$

which is equal to $4(1-\lambda)$. Each of the above ratios has a simple binomial variance and the variance of λ is one-sixteenth of the sum of the variances of each of the eight ratios, the covariance term being zero as eight non-overlapping totals are being considered.

For the female data $\lambda = 3{\cdot}813\pm1{\cdot}876$ per cent., and for the male, $\lambda = 6{\cdot}910\pm1{\cdot}959$ per cent.

In table 9 the six recombination values are given with standard errors.

These recombination values show a striking sex difference, the females almost invariably giving greater recombination values than the male, except over the entire segment *fz-py* where 49·32 per cent.

recombination is obtained in the females and 49·44 per cent. in the males. The *py-ln* value agrees with Fisher (1953), although the large difference between the sexes that he obtained is reduced a little. In the other long segment, *Sp-fz*, the sex difference is significant, but not as striking as for the *py-ln* segment. The figures obtained here agree reasonably with the figures of Snell, Dickie *et al.* (1954).

The *ln-Sp* values are smaller than those given by Snell, Dickie *et al.* (1954), and very much smaller than the first data given for this linkage by Ingalls (reported in Snell, Dickie *et al.*). In these first

TABLE 9

Recombination values

Segment	Modes of gamete formation	Recombination values	
		Females	Males
py-ln (3)	$s+u+v+w$	37·9±0·98	23·9±1·06
ln-Sp (2)	$r+t+v+w$	5·3±0·42	4·6±0·41
Sp-fz (1)	$q+t+u+w$	40·2±0·92	33·0±0·93
py-fz (123)	$q+r+s+w$	49·3±0·97	49·4±1·06
py-Sp (23)	$r+s+t+u$	40·4±0·97	27·6±1·06
ln-fz (12)	$q+r+u+v$	43·0±0·93	35·9±0·95

data, *Sp* classification was complicated by piebald (*s*) segregation and recombination values were 9·8±7·8 for females, and 9·7±4·5 for males. Any misclassification would almost certainly give spuriously high values for a small segment. It seems probable that the figures reported here are nearer to the true values as very little *Sp* misclassification was detected.

However, the novel feature of these data is the equality of *py-fz* recombination values in both sexes when compared with the lower values obtained for males in the segments making up the *py-fz* segment.

5. DISCUSSION

5.1. *Interference*

A simple measure of interference is the Kosambi coefficient K (Owen, 1950). In a backcross experiment involving two segments AB and BC, let y_1, y_2 be the recombination values for each segment, y_{1-2} the recombination value for the entire segment AC, and y_{12} the frequency of simultaneous recombination in both segments, then K is given by—

$$\frac{y_{12}}{2y_1y_2y_{1-2}}$$

Kosambi (1944) has proposed the relation $2y = \tanh(2x)$ to relate the map distance x, and the recombination value y between two loci. When K = 1, Kosambi's relation is satisfied. In practice, for

Drosophila (Owen, 1948), and maize (Parsons, 1957*c*), K is found to exceed unity in the proximal regions of long chromosome arms, and drop below unity in the distal regions. Owen (1948, 1949) has expressed x and y in terms of a metric u in which interference is uniform, and has postulated $f(u) = 4ue^{-2u}$ in which the intercept length is distributed as $\frac{1}{4}\chi^2_4$ as an adequate fit to the observed data. Assuming this form for the interference function, K = 1 at the metrical mid-arm of long chromosomes. Calculation of K values will therefore provide a valuable guide to the nature of interference along the chromosomes. In table 10, K values are given for the four possible triplets of loci for contiguous segments with their standard errors calculated from the formula given by Owen (1953*b*).

TABLE 10

Kosambi coefficients

(Order *fz-Sp-ln-py*)

Triplet	Female	Male
fz-ln-py	0·982 ± 0·046	0·611 ± 0·051
fz-Sp-py	0·976 ± 0·046	0·620 ± 0·051
fz-Sp-ln	0·694 ± 0·105	0·782 ± 0·156
Sp-ln-py	0·862 ± 0·124	0·743 ± 0·211

There is a major sex difference reflecting that of the recombination values. In the male data K values are consistently lower than those for the female data. The data from female heterozygotes, with the exception of *fz-Sp-ln*, give K values not significantly lower than unity, whereas all values for the male data are well below unity. The K values for *fz-ln-py* and *fz-Sp-py* are based on a large number of double recombinants and are consequently more accurate than K values based on triplets which include the short segment *Sp-ln*. *Sp* misclassification, if present, would give spuriously high K values due to the increase of the double recombinant class by misclassification of single or non-recombinants as double recombinants. In these data, triplets with *Sp* included do not give higher values, an observation which suggests that *Sp* misclassification is negligible.

Taking the male data by itself, all K values are below unity. On existing data, in organisms other than mice, this observation is indicative of a centromere situated outside the segment under discussion or close to one of its ends but just within it. Further investigation of the male data does not allow a decision to be made as to which end may be involved. In the female data, all K values are close to unity except for the segment *fz-Sp-ln* for which K = 0·694 ± 0·105. As K values are expected to decrease with distance from the centromere, it seems probable that the centromere is near to *py* rather than near to *fz*, and in view of the low K values for the male data, the centromere is probably outside the whole segment.

The data, however, scarcely in this case afford a decision for it is possible that interference could be generally more intense in mice than in other organisms studied so far. This is supported by Owen's (1953) data and Wallace's (1957) data for linkage group V.

Additional evidence can be drawn from the sex differences in recombination. Changing the interference metric will result in a variation of the recombination fraction between loci, the greatest variation occurring in the centromeric regions where the metric is most sensitive to change. If the sex differences can be explained as due to a different metric for each sex, there would be a large sex difference in recombination near the centromere decreasing with distance from the centromere. In the data under analysis, the greatest sex difference is for the segment *py-ln*. Hence, on this hypothesis, it can be said that the centromere must be located near *py* which is in agreement with the interference data.

Further evidence on the sensitivity of centromeric regions to changes of metric comes from studies on the effect of environmental and genetic variables on recombination, if again it is assumed that such variables operate by varying the metric. Temperature (Plough, 1917), age (Bridges, 1915), X-rays (Muller, 1925), and heterozygous inversions (Schultz and Redfield, 1951) have been shown to produce a variation in recombination near the centromere but very little elsewhere.

5.2. *Mapping the chromosome*

Using Owen's $\frac{1}{4}\chi^2_4$ distribution for the interference function, Fisher (1951) expressed the frequencies of all recombination classes for any number of linked loci on a finite arm in terms of a combinatorial scheme based on four simple functions α, β, γ and δ which he termed segmental functions and are tabulated in Fisher and Yates (1953).

Segments are assigned various metrical lengths and from these, expected recombination values can be found using segmental functions, and then compared with observed with a χ^2 test. From the best fitting metrical lengths, the values of the map distance x between two loci may be calculated using the formula

$$x_{12} = \left[u \tanh 2T + \frac{\cosh 2(T-2u) - \cosh 2T}{4 \cosh 2T}\right]_{t_1}^{t_2}$$

where T is the metrical length from centromere to terminus, and t_2 and t_1 are the metrical distances of the two loci from the centromere such that $t_2 > t_1$ (Owen, 1948).

For the data from the male heterozygotes, the following map was obtained :

	C	*py*	*ln*	*Sp*	*fz*	T
metrical length		90	26	5	30	25
map distance		66·33	26·98	5·45	36·23	40·71
recombination			23·93	4·62	32·96	

where C represents the centromere, and T the terminus. This map

gave $\chi^2_6 = 6{\cdot}43 (0{\cdot}3 < P < 0{\cdot}5)$ for a goodness of fit test. The arm is 175·70 cM long, approximately one-third of this distance being from the centromere to *py*. This long proximal segment is reflected in the low K values obtained from the male data. Furthermore, the map distances between the markers *py*, *ln*, *Sp* and *fz* are close to the observed recombination values, as at this distance the effect of the centromere is lost.

For the female data, the following map was obtained :

	C	*py*	*ln*	*Sp*	*fz*	T
metrical length		30	52	5	50	50
map distance		12·54	45·68	4·91	52·20	71·46
recombination			37·93	5·29	40·22	

This map gave $\chi^2_7 = 22{\cdot}62$ ($P < 0{\cdot}01$) for goodness of fit, which is clearly highly significant. In this case the total length of the arm is 186·79 cM which is only 10·09 cM longer than the map for male data. However, the K values for females are higher, and the C-*py* segment much shorter than for the males. Assuming the $\frac{1}{4}\chi^2_4$ metric of intercept length, a recombination value of 52·48 per cent. is expected between *py* and *fz* whereas the observed value was 49·32 per cent. The observed value is significantly different from the expected value ($\chi^2_1 = 5{\cdot}38$, $P < 0{\cdot}05$). This large discrepancy therefore accounts for the poor fit of the map obtained using segmental functions.

The female data are therefore of theoretical importance in determining when recombination can be expected to be >50 per cent. Recombination values of more than 50 per cent. have been reported in mice for the factors dilute and wavy (Fisher and Mather, 1936*a*, 1936*b*), and for sex with both shaker and wavy (Wright = Wallace, 1947). Other cases reported are those of Wellensiek (1929) in *Pisum* and Clausen (1926) in *Viola*.

In the absence of interference, y is always less than 50 per cent. and increases towards 50 per cent. as a limit. If interference were complete, then $x = y$, and the increase in y would keep pace with that in x. Observation suggests that interference is always incomplete and vanishes over long intervals, so that on a long chromosome two loci remote from each other would exhibit extremely loose linkage and would segregate showing recombination of 50 per cent. with one another. This leaves the question of less remote loci which may show recombination greater than 50 per cent. as in fact has occurred, and is predicted by the $\frac{1}{4}\chi^2_4$ metric. Data for segments likely to show recombination greater than 50 per cent. are therefore of extreme value in determining whether the $\frac{1}{4}\chi^2_4$ metric is realistic, or whether some small alteration is needed in the metric. For this reason, this experiment was set up, and the result shows that the $\frac{1}{4}\chi^2_4$ metric may need some small adjustment, although it must be pointed out that for shorter segments this metric has been shown to be a good approximation in mice, maize, and *Drosophila*.

In mice, Wallace (1947) reported a case of recombination significantly in excess of 50 per cent., a phenomenon Owen (1953*a*) has termed super-recombination. The two factors wv_2 (wavy-2) and sh_2 (shaker-2) showed respectively with sex 56·07 and 56·73 per cent. recombination and 31·06 per cent. recombination with each other. Fisher, Lyon and Owen (1947) showed that these results could be accounted for on the basis of genetical interference and this was extended by Owen (1953*a*) to take into account chromosomes of finite lengths. In this discussion Owen shows that the delicate phenomenon of super-recombination shows up the slight quantitative differences between the distribution proposed by Fisher, Lyon and Owen (1947) :

$$\frac{d}{du}\left(-\operatorname{sech}\frac{\pi\mu}{2}\right)$$

and the more convenient mimic $4ue^{-2u}$ which is distributed as $\frac{1}{4}\chi^2_4$. The data presented here for linkage group XIII fall into the small group of data which could possibly give information on super-recombination and hence test any proposed metric. That no super-recombination occurs is significant, and shows that the $\frac{1}{4}\chi^2_4$ distribution of intercept lengths is not adequate in this case.

As well as this difficulty in the female data a striking sex difference in recombination is demonstrated which indicates that each sex for linkage group XIII requires a specific but different distribution of intercept lengths in terms of which interference is uniform. This is supported by Wallace's (1957) data for linkage group V as well as a general tendency in the house mouse for recombination values to be greater in the female.

6. SUMMARY

1. Linkage data for the four genes *fz-Sp-ln-py* in the house mouse are presented for male and female quadruply heterozygous parents.

2. The data are balanced, each possible quadruple heterozygote having been made up.

3. Several preliminary tests are carried out to detect viability and manifestation disturbance and the results of these tests are taken into account when the recombination values are computed.

4. A large sex difference in recombination was found, the male values being lower than the females except for the entire segment *py-fz*.

5. The sex difference in recombination was reflected in a major sex difference in interference. The male interference data are incompatible with the centromere within the segment *py-fz*.

6. The chromosome is mapped using the segmental functions tabulated in Fisher and Yates (1953) for the $\frac{1}{4}\chi^2_4$ distribution of intercept lengths proposed by Owen. A good fit was obtained for the data from male heterozygotes but data from the female heterozygotes gave a

bad fit. On theoretical grounds the female data should have given a *py-fz* recombination of 52·48 per cent. thus exhibiting super-recombination, but the observed value was 49·32 per cent. The possibility that the $\frac{1}{4}\chi^2_4$ distribution may not be quite adequate is suggested and also the possibility of different distributions of intercept length for each sex.

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UNCLE-NIECE INBREEDING FOR AUTOSOMAL GENES

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1. INTRODUCTION

THE occurrence in South India of preferred uncle-niece marriage has been described by Aiyappan (1934). Double cousin marriage occurs much more frequently. It is intended in this paper to investigate the results of continued uncle-niece inbreeding, for the case of autosomal genes, and to compare the speed of the process with that obtained in continued double cousin inbreeding.

2. GENERATION MATRIX

It is supposed that the inbreeding process is carried on by a single mating in each generation. Then if m_t is the mating in the t^{th} generation, the female parent ("niece") in m_{t+1} is chosen at random from the female offspring of m_t, whereas the male parent ("uncle") in m_{t+1} is chosen at random from the brothers of the female parent in m_t. In order to specify the genetic situation in m_{t+1}, it is necessary to consider not only m_t but also the uncle who is to be the parent in m_{t+1}. This may be done by listing mating types, every type consisting of a mating m_t in conjunction with such an uncle. There are 9 heterogenic mating types, namely

Mating type	Frequency	Mating	Uncle
(1)	r	$aa \times Aa$	aa
(2)	s	$aa \times aa$	Aa
(3)	t	$Aa \times Aa$	aa (or AA)
(4)	u	$aa \times Aa$	Aa
(5)	v	$Aa \times Aa$	Aa
(6)	w	$aa \times AA$	aa (or AA)
(7)	x	$aa \times AA$	Aa
(8)	y	$aa \times aa$	AA
(9)	z	$aa \times Aa$	AA

The generation matrix is constructed by tracing out the consequences of the various mating types, in the usual way. In type (4), for example (fig. 1) we have in generation t

$$aa \times Aa \rightarrow \tfrac{1}{2}aa,\ \tfrac{1}{2}Aa$$

so that in the next generation the mating is

		Probability
	$aa \times Aa$	$\frac{1}{2}$
or	$Aa \times Aa$	$\frac{1}{2}$

and uncle

	aa	$\frac{1}{2}$
or	Aa	$\frac{1}{2}$

giving, in $t+1$,

		Type	Probability
$aa \times Aa$	aa	(1)	$\frac{1}{4}$
$aa \times Aa$	Aa	(4)	$\frac{1}{4}$
$Aa \times Aa$	aa	(3)	$\frac{1}{4}$
$Aa \times Aa$	Aa	(5)	$\frac{1}{4}$

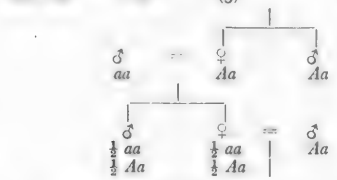

FIG. 1.—Genetic consequences of mating type (4).

In this way, the generation matrix, omitting the first row and column (which represent the homogenic mating type), is found to be

	$\frac{r_0}{4}$	$\frac{s_0}{1}$	$\frac{t_0}{16}$	$\frac{u_0}{4}$	$\frac{v_0}{8}$	$\frac{w_0}{1}$	$\frac{x_0}{1}$	$\frac{y_0}{1}$	$\frac{z_0}{4}$
r_1	1	1	2	1	1	.	.	.	.
s_1	1	.	2	.	.	.	.	.	.
t_1	.	.	.	1	2	.	.	.	.
u_1	1	.	4	1	2	1	.	.	1
v_1	.	.	.	1	2	.	1	.	.
w_1	.	.	2	.	.	.	.	1	1
x_1	.	.	2	.	.	.	.	.	1
y_1	.	.	1	.	.	.	.	.	.
z_1	.	.	2	.	1	.	.	.	1

The column divisors are shown for convenience at the head of each column, instead of being entered in the body of the matrix. The λ-matrix is obtained by subtracting from each element in the diagonal the product of λ and the corresponding column divisor. The 9×9 determinant of the λ-matrix is then reduced to an 8×8 determinant as follows. From given rows (or columns) appropriate multiples of other rows (or columns) are subtracted in order to produce a 9×9 determinant with unity as the sole element in one of the rows (or columns). On expanding by such a row (or column) an 8×8 determinant is obtained. The process is successively repeated until a 2×2 determinant is obtained which on expansion gives the characteristic equation, which is

$$8192\lambda^9 - 8192\lambda^8 - 512\lambda^7 + 768\lambda^6 - 128\lambda^5 + 304\lambda^4 + 24\lambda^3 - 4\lambda^2 - \lambda - 1 = 0$$

The real roots and at least one of the imaginary pairs of roots having been evaluated numerically, it appears that this polynomial

in λ is the product of four factors, two of which are linear, one cubic, and one quartic, as shown below.

$$(1-4\lambda)(1+4\lambda)(1+16\lambda^2-32\lambda^3)(1+\lambda+4\lambda^2+8\lambda^3-16\lambda^4) = 0.$$

There are 5 real and 4 imaginary roots, resulting from the factors indicated.

Factor	Roots
linear .	$\frac{1}{4}$, $-\frac{1}{4}$
cubic .	$0{\cdot}58982602$, $-0{\cdot}04491301 \pm i(0{\cdot}22575329)$ or $0{\cdot}23017759 \exp \pm i\ 101{\cdot}25193^0$
quartic .	$0{\cdot}92341043$, $-0{\cdot}44686647$, $0{\cdot}01172802 \pm i(0{\cdot}38900614)$ or $0{\cdot}38918298 \exp \pm i\ 88{\cdot}27313^0$

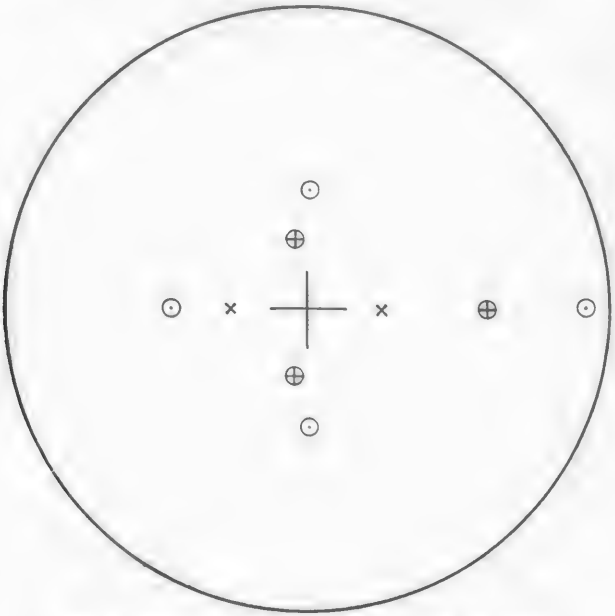

FIG. 2.—Roots plotted as points lying within the unit circle
× = linear factor, ⊕ = cubic factor, ⊙ = quartic factor.

The dominant latent root λ_D is therefore $0{\cdot}92341043$. The roots are shown diagrammatically in fig. 2, being plotted as points lying within the unit circle, the co-ordinates of a point being the real and imaginary parts of the root depicted.

3. COMPARISON WITH DOUBLE-COUSIN INBREEDING

	λ_D	$\log_e \lambda_D$	
Double-cousin inbreeding .	$0{\cdot}91964338$	$-0{\cdot}083770$	(Fisher, 1949)
Uncle-niece inbreeding .	$0{\cdot}92341043$	$-0{\cdot}079682$	

The ratio of the two logarithms is $1{\cdot}0513$, so that one generation of double-cousin inbreeding is equivalent to $1{\cdot}0513$ generations of uncle-niece inbreeding. The rate of progress towards homozygosity

thus, somewhat surprisingly, proves to be slightly slower in the case of uncle-niece inbreeding. In contrast, English law permits double-cousin marriage but forbids uncle-niece marriage.

4. LIMITING FREQUENCIES AND COMPLEXITIES

The numerical values of the frequencies and complexities corresponding to the dominant latent root are

Mating type	Limiting frequency	Absolute complexity (C)	$\log_e C/(-\log \lambda_D)$ (generations)
(1)	0·27896088	0·69496309	−4·565
(2)	0·09035953	0·75260477	−3·566
(3)	0·10958967	1·11200698	1·332
(4)	0·26559044	1·11937680	1·411
(5)	0·13919455	1·20822970	2·371
(6)	0·03537012	1·21222023	2·412
(7)	0·02733745	1·30844278	3·369
(8)	0·00741745	1·31276428	3·417
(9)	0·04617990	1·35134520	3·775
	0·99999999		

The absolute complexities may be interpreted as follows (Fisher, 1949). Suppose we have a hypothetical foundation mating type of standard (*i.e.* unit) complexity and that in such a case a given amount of inbreeding is achieved in n generations. If, starting from a foundation mating type i of complexity C_i, m generations are required to achieve the same amount of inbreeding, then

$$\lambda_D^n = \lambda_D^m C_i$$

$$(m-n) = \log_e C_i/(-\log_e \lambda_D) \text{ generations.}$$

A negative value of this ratio therefore means that the process starting from i is ahead of standard, and a positive value that it lags behind standard, by $|\log_e C_i/(-\log_e \lambda_D)|$ generations.

5. SUMMARY

The dominant latent root of a regular uncle-niece inbreeding system has been shown by the matrix method to be 0·92341043. Progress to homozygosity is slightly slower than in the case of double-cousin inbreeding. Limiting frequencies and absolute complexities have been calculated.

Acknowledgments.—I should like to thank Professor Sir Ronald Fisher for suggesting this problem and for continued encouragement. The receipt of a Medical Research Council Scholarship enabling this work to be done is gratefully acknowledged.

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GENOTYPIC CONTROL OF CHROMOSOME BEHAVIOUR IN RYE

V. THE DISTRIBUTION PATTERN OF CHIASMATA BETWEEN POLLEN MOTHER CELLS

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1. INTRODUCTION

A RECENT paper (Rees and Thompson, 1956) described the variation in chiasma frequencies in *p.m.c.* of rye inbred lines and their F_1 hybrids. It was shown that the average chiasma frequencies vary between lines and between F_1 families; they are genotypically controlled. Not all the chiasma variation, however, could be ascribed directly to genotypic differences. Part of it is not heritable and depends on environmental factors affecting chiasma formation. Such variation was detected and measured in three ways, by comparing (1) genetically identical plants within a family, (2) *p.m.c.* within the same anthers, and (3) bivalents within *p.m.c.* While the variation measured at these three levels must be determined by environmental (including cytoplasmic) fluctuations between and within plants we found that the degree of variation, that is the response to the varying circumstances, depends on the genotype. In general this was greater in the inbred homozygous lines. We concluded that the amount of variation exhibited at one level, *e.g.* that of the plant, could be partly independent of that at another, *e.g.* the cell, and also partly independent of the mean chiasma frequency. Certain predictions follow from this conclusion; in particular that the average amount of chiasma variation between *p.m.c.* within plants of one population could differ from that in another even where the average frequency of chiasma formation per plant is the same for both populations. Where populations are outbreeding differences of this kind would of course affect their gametic output in respect of recombination, and hence their properties of variability. Analyses making use of F_2 and other data enable us to test this prediction and to inquire further into the factors affecting the variation within individuals.

2. MATERIAL AND METHOD

The inbred lines used have been bred by self-pollination for more than twenty generations (*cf.* Rees and Thompson, *l.c.*). F_1's and F_2's were obtained from crosses

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between these lines. Chiasma frequency was scored in 20 *p.m.c.* in each plant, and for each plant two quantities were estimated :—

1. The *chiasma frequency*, found as the average per bivalent in the 20 cells.
2. The *cell variance* is measured by the variance—with 19 degrees of freedom—between the *p.m.c.* in chiasma frequency per bivalent.

The variation between cells could of course have been expressed as the standard deviation. We found in fact that calculations, whether based on variances or on standard deviations, gave essentially the same results.

TABLE 1

The averages of plant chiasma frequencies and cell variances in inbred, F_1 and F_2 families, with approximate standard errors. The numbers of plants scored are in brackets

	1954		1955	
	Chiasma frequency	Cell variance	Chiasma frequency	Cell variance
Lines				
P3 (9, 10)	1·85±0·028	0·141±0·049	1·91±0·024	0·202±0·028
P12 (10, 10)	1·71±0·055	0·226±0·086	1·78±0·085	0·240±0·091
F_1				
3×12 (8, 10)	2·07±0·034	0·148±0·054	2·07±0·025	0·100±0·032
F_2				
3×12 (33)			1·90±0·101	0·132±0·074
Other F_2's				
3×6 (15)			1·86±0·086	0·197±0·148
3×13 (20)			1·93±0·049	0·129±0·063
6×12 (20)			1·86±0·082	0·156±0·121
6×13 (29)			1·93±0·064	0·085±0·049
12×13 (15)			1·91±0·059	0·091±0·049

In this account we are concerned only with the variation between different genotypes and between cells within anthers. We have not considered the variation between plants of the same genotype or the variation between bivalents within cells.

Because individuals of different genotypes matured at different times not all plants were fixed on the same day. The effect of fixing on different days was, however, found not to influence significantly either of the characters scored. This was established by comparing different F_2 plants fixed over a period of about 5 weeks at intervals of about 5 days.

3. INBRED LINES AND F_1

Generally where the chiasma frequency is low, as for example in inbred plants, the variation between cells is high. In contrast high chiasma frequencies are associated with low cell variances in F_1 hybrids between lines (Rees and Thompson *l.c.*). The two characters therefore show a negative correlation. On the basis of evidence obtained chiefly by the analysis of a diallel cross we did, however, conclude in our previous work that the correlation between them is not complete. For further evidence concerning their inter-relations we therefore

sought data involving inbred and F_1 families which covered more than one season. It should be possible with such data to find out whether the characters reacted differently to different seasons and whether the relations between them were the same for all genotypes. We could, in other words, test for independence of response to environmental as well as to genotypic differences.

Adequate data were available for two lines, P3 and P12, and their F_1 hybrid, in 1954 and 1955. It will be recalled that the two seasons

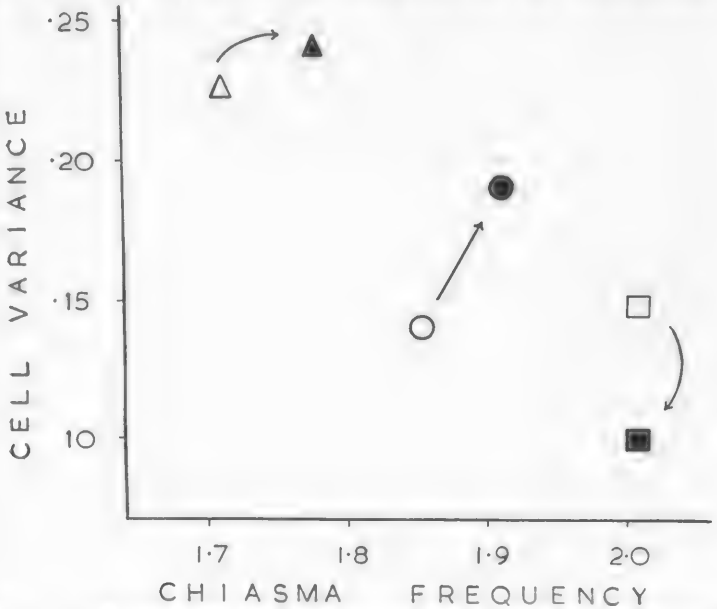

FIG. 1.—The mean chiasma frequencies of inbred and F_1 families plotted against their mean cell variances. Triangles = P12, Circles = P3, Squares = 3×12. Open symbols for 1954, blocked in for 1955. Explanation in text.

were very different, 1954 being wet and cool and 1955 hot and dry. From the plant chiasma frequencies and cell variances the averages for the lines and F_1 were calculated for both years. These, along with their standard errors, appear in table 1.

The variation between the families. In fig. 1 the family mean cell variances are plotted against the mean chiasma frequencies. The graph clearly reveals in the first place a general trend in both years, *viz.* a decreasing cell variance with increasing chiasma frequency. The regression (cell variance on chiasma frequency) of these family means is significant ($P = <0{\cdot}001$)—as shown in the analysis of variance (table 2); there is no doubt of a negative correlation.

The regression on chiasma frequency does not, however, account for all the variation in mean cell variances. This we conclude from

the fact that the item for residual variation is significant ($P = <0{\cdot}01$). Two causes could account for this residual variation (1) a curvilinear relation between cell variance and chiasma frequency, or (2) some degree of independence between the two characters. Now there is no significant heterogeneity of regressions within the families and furthermore there is no suggestion of a significant difference between the joint regressions within families ($b = -0{\cdot}331$) and the regression of means ($b = -0{\cdot}306$). On the basis of these two facts we can rule out curvilinearity as contributing other than a negligible amount in this case to the residual variation. We therefore conclude, and confirm

TABLE 2

The joint regression analysis of cell variance on chiasma frequency in P3, P12 and their F_1 in 1954 and 1955

Item	SS	N	MS	VR	P
Within families					
Joint regression	0·013	1	0·0134	3·38	0·20-0·05
Heterogeneity of regressions	0·0054	5	0·0011	...	...
Between families					
Regression of means	0·0998	1	0·0998	25·12	<0·001
Residual variation	0·0482	4	0·0121	3·03	0·05-0·01
Error	0·1788	45	0·0040	...	...
Total	0·3457	56			

our previous findings, that to some degree cell variances and chiasma frequencies vary independently of one another.

Before attempting to explain the genetical basis for this independence it is first necessary to inquire further into the variation in expression of the two characters in relation to changing environment and genotype. It is clear from these and earlier data that differences in both chiasma frequency and cell variance are genotypically determined. The present results also reveal that seasonal differences must affect their expression. Thus in P3 both chiasma frequency and cell variance are significantly higher in 1955 than in 1954 ($P = <0{\cdot}01$).* The same is true for P12 although the increase in neither character is significant. In the F_1 the chiasma frequency remains constant, whereas the cell variance is seen to be significantly *lower* in 1955 ($P = <0{\cdot}01$). It is in fact evident not only than an environmental as well as a genetic component contributes to the variation but also that the characters responded differently to environmental (seasonal) change in different genotypes.

* For these and similar comparisons the error sums of squares are based on the variation within the particular pair involved. This is more appropriate than an over-all sum of squares because different family means have widely and significantly different standard errors (see table 1).

The question to consider now is whether the variation in chiasma frequency and cell variance that is independent of their overall negative correlation is to be attributed to genotypic or to environmental causes. We shall see that both causes most probably are responsible. For example, the effect of season is to increase significantly *both* the chiasma frequency and the cell variance in P3 in 1955 in comparison with 1954 (fig. 1). These changes in the two characters due to season are *positively* correlated : they are independent of the negative correlation which applies to them generally. Two facts suggest that the varying inter-relations of chiasma frequency and cell variance depend also on the genotype, (1) the cell variance in P3 is lower than in 3 × 12 in 1954, despite the markedly lower chiasma frequency of P3 : (2) in 3 × 12 the cell variance, as in the inbred lines, is different between years, whereas (*cf.* the inbred lines) the chiasma frequency is unchanged. It must be noted, however, that these last two comparisons, in one respect, do not provide entirely satisfactory evidence. In the first place, although the cell variance of P3 in 1954 is lower than that of 3 × 12 it is not significantly so, and in so far as the mean cell variances are not known exactly the true means could, unlikely though it may be, show a negative correlation with the chiasma frequency. Secondly, while the estimated mean chiasma frequencies are the same for the two years in 3 × 12 the true means might possibly not be. More evidence confirming the role of genotype in controlling the inter-relations of the two characters is therefore desirable. Such evidence will be presented in the later sections.

The variation within families. The analysis of variance provides overall no evidence of a correlation between chiasma frequency and cell variance within the families considered as a group—the item for the joint regression is not significant. When, however, regressions are calculated for the families individually a significant negative correlation is found within P12. There is no indication from the analysis that the regressions within the families are significantly different from one another, and no evidence therefore in these results to suggest that genetic differences between the families affect the correlations within them.

4. PARENTS, F_1 AND F_2

In 1955, 33 plants of an F_2 derived from a cross between P3 and P12 were fixed over the same period as the parent lines and the F_1. We have investigated the relations between chiasma frequency and cell variance in these four families and made comparisons between them. In particular, we sought evidence of genotypic variation affecting the relations between the characters. This has been done, as in the previous section, by means of a joint regression analysis of cell variance on chiasma frequency in the parents, F_1 and F_2. The analysis is given in table 3.

Within families. In the inbred lines and F_1 the variation is of course non-heritable, whereas the F_2 includes a heritable component in addition. Within the families, as the analysis shows, there is, overall, a correlation between the characters; the joint regression is significant ($P = <0{\cdot}001$) and it is negative. There is no evidence that the regressions within the homogeneous families (parents and F_1) are different from that within the F_2. Neither, as shown earlier, are the regression slopes within the homogeneous families significantly different from one another. Thus, from the comparisons of the regressions within families we can demonstrate no variation that could be attributed to genotypic differences between them.

Between families. Evidence of genetic differences affecting the correlation between cell variance and chiasma frequency comes from

TABLE 3

The joint regression analysis of cell variance on chiasma frequency in P3, P12, their F_1 and F_2 in 1955

Item	SS	N	MS	VR	P
Within families					
Joint regression . . .	0·1119	1	0·1119	42·39	<0·001
Heterogeneity of regressions .	0·0088	3	0·0029	1·11	...
Between families					
Regression of means . .	0·0810	1	0·0810	30·68	<0·001
Residual variation of means .	0·0607	2	0·0303	11·49	<0·001
Error	0·1452	55	0·0026		
Total . . .	0·4076	62			

a consideration of the variation between the means of the families. The regression of means is significant ($P = <0{\cdot}001$), and negative, but, as shown in the analysis, there is again a significant variation in cell variance that is independent of the regression on chiasma frequency ($P = <0{\cdot}001$). Since there is again no indication of curvilinearity, we conclude that to some degree the cell variance varies independently of the chiasma frequency, and must, furthermore, be genotypically determined.

Another comparison suggests more precisely how this variation could have arisen. When the regression of cell variance on chiasma frequency is calculated for the pooled data from the parents and the F_1, we should, subject to error variation, obtain a good estimate of the change of cell variance over the range of chiasma frequencies exhibited by the three genotypes. The F_2 chiasma frequencies, as a result of recombination in F_1, and also of environmental fluctuations, cover a considerable part of this range. If we assume no heritable variation affecting the correlation between the characters, we should expect the F_2 chiasma frequencies to be associated with cell variances

not significantly different from those in parent and F_1 plants with comparable chiasma frequencies. The regressions for the F_2 and for the parents and F_1, are plotted in fig. 2. The regression co-efficients are not significantly different, *i.e.* there is no evidence that the rate of change of cell variance on chiasma frequency is different in the two groups. The graph does, however, suggest that for a given

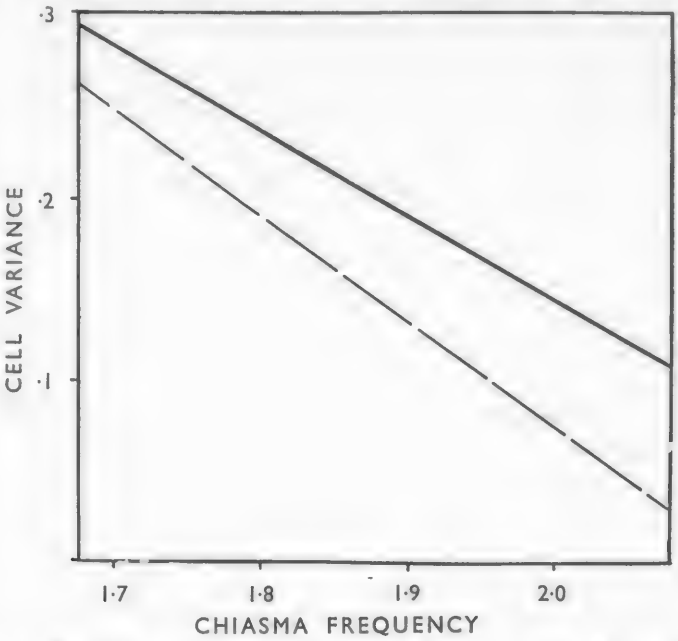

FIG. 2.—The regressions of cell variance on chiasma frequency in plants of P3, P12 and their F_1 (solid line) and their F_2 (broken line) in 1955. For the same chiasma frequencies the F_2 cell variances are lower.

chiasma frequency the cell variance in F_2 is lower. An analysis of variance shows the difference to be significant ($P = <0{\cdot}001$). It may well be, therefore, that one generation of inbreeding by self pollination, which reduces the mean chiasma frequency considerably, has a disproportionately smaller effect on the cell variance (which increases with inbreeding, *cf.* Rees, 1955).

5. A COMPARISON OF F_2 FAMILIES

In so far as genetical adjustments in chiasma frequency and cell variances could be expected in natural populations, they would be effective in relation to recombination and the release of heritable

variation in heterogenic populations of outbreeding species. In order to investigate somewhat comparable populations in rye we grew six F_2 families in 1955. These were derived from self-pollinating the F_1's from a diallel cross involving lines P3, P6, P12 and P13 (see Rees and Thompson *l.c.*). They include the 3×12 F_2 described in the previous section. Altogether 132 plants were scored and the data are presented in table 1.

As for the previous data, we need to consider and compare the rate of change of cell variance on chiasma frequency within and between the different families. Once again the comparisons were made by a joint regression analysis of variance (table 4).

TABLE 4

The joint regression analysis of cell variance on chiasma frequency in six F_2 families grown in 1955

Item	SS	N	MS	VR	P
Within families					
Joint regression . . .	0·2906	1	0·2906	56·1	<0·001
Heterogeneity of regressions .	0·0201	5	0·0040	...	...
Between families					
Regression of means . .	0·1169	1	0·1169	22·58	<0·001
Residual variation of means .	0·0439	4	0·0110	2·12	0·10-0·05
Error	0·6215	120	0·0052		
Total . . .	1·0930	131			

It will be seen firstly that the joint regression within families is significant, the cell variance being negatively correlated with the chiasma frequencies. Secondly, there is no significant heterogeneity of the regressions in the different families, and no evidence therefore that the rate of change of cell variance on chiasma frequency varies with the different genotypes.

We see also, from the analysis, that the regression of the means of the families is significant ($P = <0{\cdot}001$). The correlation is negative and there is no doubt that families with the higher chiasma frequencies tend to have lower cell variances. We can next look for evidence of variation in the characters that is independent of this correlation. From these F_2 data the evidence is not so conclusive as from the results previously analysed. The mean square for the residual variation of means is not significant. It does, nevertheless, approach the 5 per cent. level of significance, and we can, in view of our previous evidence, reasonably assume that this in part reflects independent variation of the two characters. An examination of fig. 3, in which the mean chiasma frequencies of the families are plotted against the mean cell variances, supports this assumption. Thus it will be seen that each of two pairs of families have virtually identical chiasma

frequencies, 6×12 and 3×6 with means of 1·86 and 6×13 and 3×13 with means of 1·93. We note, however, that the mean cell variances of the families within each pair differ considerably, despite the similarities in their chiasma frequencies. Between 6×13 and 3×13 the difference in cell variances, which are 0·085 and 0·129 respectively, is highly significant (P = <0·01). This kind of variation between populations is exactly what we should expect where the genotype can bring about independent adjustments in cell variances and chiasma

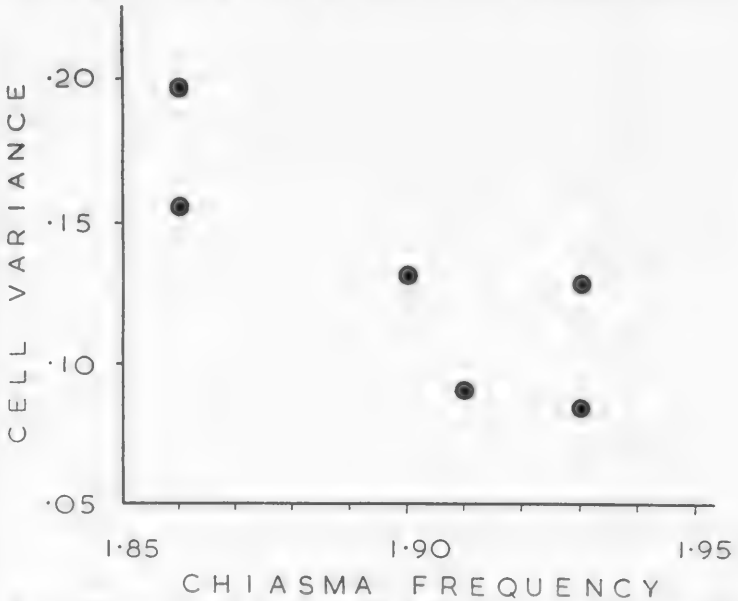

FIG. 3.—The mean chiasma frequencies of F_2 families plotted against their mean cell variances. The two families with means of 1·93 have significantly different cell variances.

frequencies. In these populations the amount of recombination is the same but the pattern of distribution within plants different.

6. THE PHYSIOLOGICAL MECHANISM

One further aspect of the variation in cell variance and chiasma frequency needs to be considered, *i.e.* the physiological mechanism governing the relations between them. In our previous work we concluded that the negative correlation found between the two characters within inbred lines was determined as follows :

1. There is an upper limit to the number of chiasmata per cell within an anther. The upper limit was deduced from investigating the distributions of *p.m.c.* chiasma frequencies within anthers (see also Mather, 1936).

FIG. 4.—The distribution of chiasma frequencies in 20 *p.m.c.* in P3 plants grown in 1954 and 1955. Except for alternation of plant distributions from different years the arrangement of histograms is random. Histograms for 1955 are blocked in. The upper limit per cell is higher in 1955.

2. It follows that the greater the cell variance the more cells will deviate from this limit. The result will be lower chiasma frequency, and *vice versa*; hence the negative correlation.

It was also suggested that one way in which the cell variance could change independently of the chiasma frequency in these lines was by alteration of the upper limit. Using our present data, a comparison of the chiasma frequency distributions of P3 plants in 1954 with those in 1955 (fig. 4) indicates that the upper limit can indeed alter; it is higher in 1955 than in 1954. The increase in the upper limit would explain, on our view, why the average chiasma frequency of P3 in 1955 is higher, despite an increase in cell variance.

The negative correlations within the F_2 families are essentially similar to those within inbred lines. They can be explained on the basis of the same upper limit mechanism, although within the F_2 the variation in part would be heritable variation. Further, the partial independence of chiasma frequency and cell variance in the F_2, as in the inbred lines, could be equally well accounted for by changes in the upper limit. While the change in upper limit described in P3 was the result of environment, there is every reason to suppose that a similar phenotypic change in the F_2 could be determined by the genotype.

7. SUMMARY AND CONCLUSIONS

Previous work has shown that the average plant chiasma frequency is genotypically controlled, and also that the genotype influences the amount of non-heritable variation in chiasma frequency between *p.m.c.* within a plant. The investigations described confirm these results. They also show that both characters respond to environmental (seasonal) changes, and that the response may be different in different genotypes.

Generally in rye plants with high chiasma frequencies the variation between *p.m.c.* is relatively small, and *vice versa*. This negative

correlation may be due to some degree of pleiotropy or to linkage of genes controlling the two characters. Evidence is produced that the correlation is not, however, invariable : it can be altered by environmental and genetic changes. The evidence may be summarised as follows :

1. In an inbred line seasonal change increases *both* the chiasma frequency and the cell variance (which measures the variation between *p.m.c.*). These changes are *positively* correlated. In this case the increased average chiasma frequency is associated with a higher " upper limit " to the chiasma frequency per *p.m.c.* within an anther.
2. In an F_1 family grown under the same conditions the cell variance decreased significantly, whereas the chiasma frequency remained constant. Compared with the inbred parent lines and their F_1 the comparable chiasma frequencies of the F_2 were associated with significantly lower cell variances.
3. Two F_2 families with identical mean chiasma frequencies differed in their cell variances. Another pair of families with the same chiasma frequencies also differed considerably in cell variance, although not significantly in this case.

We conclude, therefore, that even within a species the pattern of recombination, particularly in respect of the relation between the average per individual and the variation between cells within individuals, may be adjusted genotypically. It is important to know whether, and under what circumstances, the patterns of recombination both between and within individuals vary between natural populations. There is already some evidence from *Drosophila* populations (Carson, 1954) that the average varies from one locality to another.

Acknowledgments.—We are indebted to Professor K. Mather, C.B.E., F.R.S. for his criticism and advice, and to Mr K. B. Parry and Miss C. Boulding for photographing the diagrams.

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THE NATURE OF THE BREEDING SYSTEM IN THE FIELD BEAN (*V. FABA* L.) AND ITS RELATIONSHIP TO BREEDING FOR YIELD

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THE field bean is one of the very few high protein grain crops which can be grown successfully on farms in Britain, and for this reason it is of great importance as a source of concentrated protein for livestock. However, in recent years it has become an extremely uncertain and unprofitable crop, and despite its potential usefulness is regarded with some mistrust. Over the past seventy years the acreage has fallen from approximately 450,000 acres to under 100,000 acres, and, according to the Agricultural Statistics for 1882-1953, the average yield per acre has shown a slight decrease over the last twenty years. In comparison with cereal grain crops the yields per acre are generally poor.

The importance of the breeding system in relation to grain production has received little attention hitherto, and the precise nature of the breeding system itself has never been described. Until this situation is remedied, attempts at plant improvement can proceed only with difficulty.

Charles Darwin (1900) stated that bean plants which had been protected from insects gave one-third the number of pods which are produced by unprotected plants; it was concluded therefore that *V. faba* was a naturally cross-pollinating species. Many other investigators remarked on the entomophilous nature of the bean flower and supported the suggestion that it was an outbreeding species. On the other hand, it has been shown that the proportion of cross-pollination which takes place in the field is less than 50 per cent., and more often in the region of 30-40 per cent. (Fyfe and Bailey, 1951; Picard, 1953), these results have prompted many to consider beans as generally self-fertilising (Reisch, 1952).

A study of the cytology and floral mechanism in *V. faba* undertaken over the last few years has suggested an explanation of the apparently contradictory results previously obtained, and the implications of these findings for possible methods of improvement of the crop are discussed below.

The plant material used in these studies formed part of a *Vicia* collection, acquired from various sources. It consisted of 28 large-seeded and 18 small-seeded forms,

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but as the two types were indistinguishable cytologically and morphologically they were dealt with as one.

Pollen grain fertility was estimated on the basis of stainability with strong aceto-carmine/glycerol mixture. The glycerol was added to the aceto-carmine in equal proportion to reduce the movement of empty pollen grains to the edge of the coverslip.

Germination of pollen grains was carried out by the hanging drop method, with the ring unsealed and placed in a desiccator with a mixture of glycerine and water to adjust the humidity to approximately 50 per cent. (Darlington and La Cour, 1947). Three different culture media were used namely, 15 per cent. sucrose, style extract, and 1 part style extract to 1 part 15 per cent. sucrose. The style extracts were made by grinding a small number of styles in a drop of sterile distilled water using a tube and pestle (Glick, 1949).

Data on selfing individual plants with and without tripping were obtained by bagging the plants in fine cheese-cloth bags or in cheese-cloth cages.

1. CYTOLOGICAL OBSERVATIONS

A cytological survey of some 23 types of *V. faba* was undertaken using both root-tip and anther material. Fixations were made in Carnoy (6 : 3 : 1), and aceto-carmine squash preparations were exclusively used. At mitosis, no irregularities were observed ; the 12 chromosomes appeared quite normal at metaphase, with the six pairs easily recognisable. Five pairs of chromosomes have a subterminal centromere, but the sixth pair is very much larger with a median centromere and a subterminal secondary constriction. This latter pair of chromosomes are the *M* chromosomes of Maeda (1930).

During meiosis, the mean chiasma frequency per bivalent observed in normal metaphases was $3{\cdot}18 \pm 0{\cdot}05$ ($5{\cdot}83 \pm 0{\cdot}15$ for the *M* chromosome), a comparatively high figure from the point of view of the recombination index of the plant (Stebbins, 1950). Throughout the material investigated, normal metaphases (plate 1, fig. 1) were difficult to find, the frequency of irregularities which were observed being extremely high.

All the types so far examined showed abnormalities to a greater or lesser degree and under different environmental conditions, some of which appeared as early as diakinesis, but in most cases it was not until late metaphase I that they became obvious. However, when irregularities appeared during diakinesis they usually consisted of the stickiness often associated with fragments of varying size. The fragment could sometimes be associated with breakage of the large *M* chromosome (text-figs. 1, 2 ; plate I, figs. 6, 8) possibly at the weak points which exist in the heterochromatic regions as demonstrated by McLeish (1953). At metaphase, an upset in nucleic acid metabolism was inferred from overcontraction and more extreme forms of stickiness, but these phenomena, as stated previously, were comparatively rare (plate I, figs. 4, 5).

At late metaphase/early anaphase, nearly all the cells in affected anthers looked abnormal. Uncoiling of the chromosome thread was very prevalent, and this was often associated with precocious separation

of daughter centromeres and further fragmentation (plate I, fig. 9). This precocious separation of daughter centromeres frequently resulted

TEXT-FIG. 1.—Diakinesis showing heterochromatic fragment resulting from breakage during a pre-meiotic mitosis.

TEXT-FIG. 2.—Metaphase showing overcontraction of the chromosomes and a fragment resulting from breakage of the *M* chromosome.

in the formation of ring configurations presumably due to the continued association of opposite daughter chromatids (plate I, fig. 7). A normal anaphase arrangement followed by telophase did not occur; instead, the chromatids fell apart during late metaphase although they largely separated into two groups. At times, however, the only

TABLE 1

Frequency of cytological irregularities in population plants of V. faba

Irregularity	Per cent. of total cells in plants				
	1	2	3	4	5
Bridge and fragment . .	3·4	...	...	...	...
Bridge only : anaphase I . .	11·0	3·2	1·3	...	...
Bridge : anaphase II . .	1·3	...	...	...	1·4
Fragments only . . .	24·8	46·8	5·4	13·4	4·3
Per cent. total aberrant cells .	40·5	50·0	6·7	13·4	5·7

visible abnormalities were the bridges and fragments produced at anaphase.

Rees and Thompson (1955) have shown that bridges and fragments which occur in high frequency most probably result from splitting errors and genetically-controlled chromosome breakage. None the less, the possibility remains that at least some of the bridges which were accompanied by fragments may result from inversion heterozygosity (text-figs. 3, 4). It was found that the *M* chromosome was frequently involved in bridge formation as well as another small chromosome (text-figs. 5, 6; plate II, fig. 4), a fact which appears to confirm the suggestion that breakage and splitting errors are responsible here for many of the bridges at anaphase. Another feature of note

was that in the artificially-produced autotetraploid, the irregularities proved to be of a more extreme form, which further emphasises their gene-controlled nature.

TEXT-FIGS. 3 and 4.—Anaphase bridges with accompanying fragments.

Pollen fertility in the flowers which reached maturity was between 80-90 per cent. although in some cases half the anthers appeared to be empty in an otherwise fertile flower.

TEXT-FIGS 5 and 6.—Anaphase bridges involving the *M* chromosome but without fragments.

2. FLORAL MECHANISM

An examination of the floral mechanism to obtain more critical information on the actual breeding system of beans showed the necessity for tripping to effect efficient seed production as originally demonstrated by Reisch (1952).

Tripping entails the release of the stigma and style from the keel of the flower where it has been retained under pressure. In the bean

this mechanism results in the breakage of tiny papillæ on the stigma with consequent discharge of their contents. In lucerne also, tripping has been shown by Armstrong and White (1935) to result in similar damage of the stigmatic surface with the liberation of stigmatic

TABLE 2

The effect of tripping on pod-setting in V. faba

	Number of		Per cent. flowers forming pods
	Flowers	Pods	
No tripping-selfing . .	215	5	2·3
Tripping-selfing . . .	227	29	12·4
Open-pollinated . . .	8463	1957	23·1

contents, thus enhancing germination and growth of foreign pollen as distinct from self pollen. A similar phenomenon appears to exist in beans as indicated by pollen germination tests conducted in culture media to which had been added aqueous style extracts. These tests were of a preliminary nature, but the results indicate that foreign pollen germinated and grew better than self pollen in the same style

TABLE 3

Pollen germination after 19 hours in artificial culture media

Culture medium	Pollen from the same plant		Pollen from different plant	
	Per cent. germination	Pollen-tube growth	Per cent. germination	Pollen-tube growth
Style extract . .	18·9	+	41·1	++
Style extract+sucrose	30·9	++	58·4	+++
15 per cent. sucrose	39·7	+	36·2	+

\+ = germination beginning
++ = slight pollen-tube growth
+++ = pollen-tubes well grown

extract (table 3). The *p*H of the medium was found to be a critical factor and was adjusted to that of the style extract, in this case *p*H 6·5.

It appears from these results that the stylar contents discriminate to some degree between the self and foreign pollen present on the stigma, and thus ensure cross-fertilisation.

On selfing the pod-setting capacity of the plants was poor so that even when artificial tripping was complete a low seed set was obtained. The value of pod-setting for individual plants varied a great deal,

which suggests that in addition to the mechanical and pollen-tube selection for cross-fertilisation, some measure of genetic self-incompatibility appeared to exist.

3. FLOWER FORMATION AND SEED-SETTING IN THE FIELD

A study of pod formation in 10 spring varieties of field beans was undertaken in order to assess the relative importance of flower and pod production for yield.

Seed of 10 varieties was germinated in boxes in March; five plants of each at the seedling stage being planted 2 ft. apart into

TABLE 4

Mean values for various components of yield in spring beans 1954

Variety	Mean number for				Mean number per plant			Per cent. flowers forming pods
	Days to flowering	1st flower node	1st pod node	Height (cm.)	Tillers	Flowers	Pods	
A. FM16 .	98·9	8·6	8·9	74·0	5·3	198	25·5	14·0
B. Fm14 .	86·8	7·4	7·9	82·2	3·5	229	38·6	17·0
C. Fm9 .	95·9	7·5	7·9	72·3	3·1	154	32·5	20·4
D. Fm12 .	101·7	8·3	8·5	71·8	2·9	164	38·8	23·2
E. Fm13 .	95·1	8·0	9·5	72·6	2·4	163	26·9	12·3
F. Fm11 .	97·3	7·9	8·3	73·3	2·6	134	28·7	22·8
G. Fm10 .	96·3	7·6	7·7	69·7	2·8	146	26·7	19·6
H. Fm4 .	80·9	5·5	6·6	47·4	3·4	55	22·6	33·2
I. FM4 .	86·5	6·3	6·7	59·2	4·1	66	16·7	30·9
J. FM5 .	92·5	7·2	8·0	64·2	2·6	138	25·4	19·8
Total mean	93·2	7·4	8·0	68·7	3·3	145	28·2	21·3
S.E. . .	±1·2	±0·3	±0·4	±3·2	±0·5	±22	not sig.	...

replicated rows 2 ft. wide. The period from germination to the opening of the first flower was measured in days, and was fairly consistent in each variety. Small tags of plastic wire were used to mark the flowers counted from week to week, only the total number being recorded; small aborted flowers were ignored, since the comparison required was that of the total functional flowers in relation to the actual number of pods set.

Conditions during the summer of 1954 were such that a high percentage of seeds in the lower pods germinated while still attached to the plant, and frequently when the pods were green. Seed-shedding took place in certain plants due possibly to alternating rainy and dry spells, so that between 40 and 50 per cent. of the total pods were not harvested, and about 20 per cent. of the plants did not survive until harvest as a result of damage by wind and mechanical breakage. Mean value per plot was used in the data, therefore as only individual plants in each plot were lost they did not materially affect the analysis (table 4).

The data suggest that pod number is largely, though not completely, independent of number of flowers; however, once a sufficient number of flowers is produced pod formation appears to be influenced by other factors. Visitation by insects is random, and it would seem that

TABLE 5

Mean values and mean squares for yield and its components for once-grown versus *original seed, 1955*

Stock no.	Source of seed	Mean											
		Number of Pods			Number of seeds per pod			Seed size. Wt. of 100 grains (gm.)			Grain yield (gm.)		
		M_0	M_1	Mean	M_0	M_1	Mean	M_0	M_1	Mean	M_0	M_1	Mean
1	Once-grown	54·0	47·3	50·7	3·19	2·95	3·07	43·0	41·2	42·1	57·7	41·9	49·8
	Original	52·3	48·7	50·5	3·07	3·01	3·04	54·7	53·5	54·1	77·1	66·3	71·7
	Mean	53·2	48·0	50·6	3·13	2·98	3·06	48·9	47·4	48·2	67·4	54·1	60·8
2	Once-grown	17·1	23·3	20·8	2·31	2·51	2·41	87·8	74·3	81·1	27·8	39·7	33·8
	Original	12·9	16·0	14·5	2·04	2·07	2·06	65·5	76·0	70·8	12·1	21·4	16·8
	Mean	15·0	19·6	17·3	2·18	2·29	2·23	76·6	75·1	75·9	20·0	30·5	25·3
3	Once-grown	45·7	49·0	47·4	2·99	3·19	3·09	39·3	40·8	40·1	45·0	48·9	47·0
	Original	49·1	50·5	49·8	3·25	3·19	3·22	45·0	46·5	45·8	51·9	51·0	51·5
	Mean	47·4	49·7	48·6	3·12	3·19	3·16	42·1	43·7	42·9	48·5	50·0	49·3
4	Once-grown	51·5	60·8	56·2	2·92	3·15	3·04	37·8	43·0	40·4	53·2	67·8	60·5
	Original	50·1	60·5	55·3	3·25	3·13	3·19	44·8	39·3	42·0	61·9	72·3	67·1
	Mean	50·8	60·6	55·9	3·09	3·14	3·12	41·3	41·1	41·2	57·6	70·0	63·8

Mean squares

Components	N				
Stocks	3	4845·14 †	3·092 †	4512·17 †	4825·96 †
Original/once-grown seed	1	18·81	0·008	43·89	416·70
Stock/seed source	3	46·27	0·225	441·70 *	1054·74
Error (*a*)	21	140·50	0·098	91·12	416·05
Fertiliser	1	134·42	0·009	24·50	244·53
Error (*b*)	24	167·45	0·101	31·56	346·45

M_0 = no fertiliser. M_1 = 5 cwt. complete fertiliser per acre.
* = significant at 1 per cent. level. † = significant at 0·1 per cent. level.

most differences in pod setting in plants may be due to their capacity for self-pollination and self-fertilisation.

4. CROSS-POLLINATION AND BREEDING METHODS

In all bean improvement programmes, the possibility exists of contamination of breeding material through cross-pollination. The extent and importance of this was investigated in 1955, by comparing the open-pollinated progeny from the 1954 experiment with the original

seed material. Four stocks, each represented by original and once-grown seed were sown in randomised pairs of rows 2 ft. wide, alternating rows receiving the equivalent of 5 cwt. per acre complete fertiliser. Examination of the whole plot data showed that there were significant differences between stocks in all four characters measured (table 5).

The stock/seed source interaction mean square in respect of seed size was significant at the 1 per cent. level, but that for grain yield was not, although presumably the same tendency was exhibited.

The mean squares relating to treatment, and treatment/stock interaction calculated on the split-plot basis were not significant. Manurial treatments, therefore, did not appear to have any effect on pod number, seeds per pod, seed size, or yield of grain.

TABLE 6

Analysis of variance of regression : once-grown seed on initial seed

	Sums of squares	N	Mean square	Variance ratio	Probability
Replication . .	765·86	3	...	...	...
Regression . .	1840·90	1	1840·9	19·8	$t_{11} = 4{\cdot}5$ Sig. at 0·1 per cent.
Error . . .	1024·13	11	93·1	...	...
Total . .	3630·89	15	...	...	...
$Y = 47{\cdot}72 + 0{\cdot}41\ (x - \bar{x})$					

Further analysis of the effect of once-grown seed was undertaken using the regression of once-grown seed on initial seed. Taking grain yield, the equation for the regression line is $Y = 47{\cdot}72 + 0{\cdot}41\ (x - \bar{x})$. This line was calculated from the varietal values over 4 replications, giving 16 pairs of values, instead of only 4 if the mean alone had been used. The significance of this regression line can be seen from table 6 where $t = 4.5$, significant at 0·1 per cent. level.

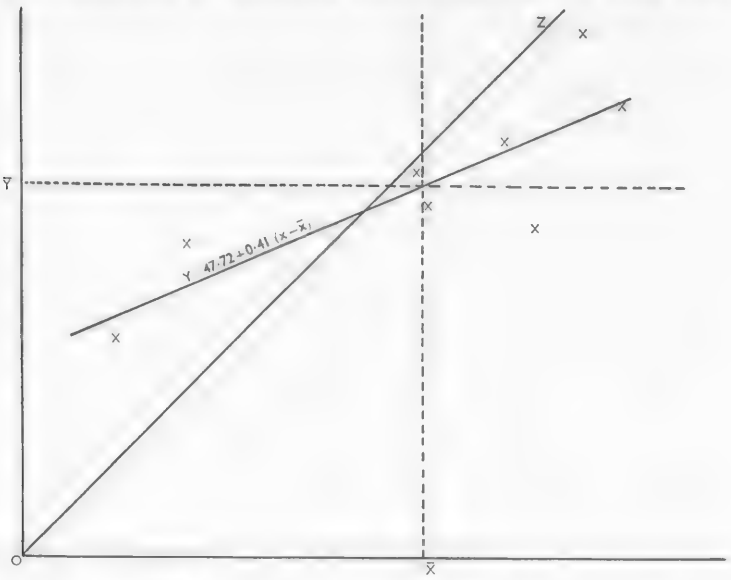
If no significant difference existed between initial material and once-grown seed, the slope of the regression line should be unity and would theoretically pass through the origin. This line is shown in text-fig. 7 marked OZ. The regression line appears to be approaching the mean of the general population and deviates considerably from the expected line OZ.

The standard deviation of the difference between the regression coefficient and the theoretical one of unity is the same as the standard error of that regression coefficient namely 0·0932, so that

$$t_{11} = \frac{0{\cdot}59}{0{\cdot}0932} = 6{\cdot}33$$

which is significant at the 1 per cent. level.

Consequently it can be said that the once-grown seed deviates significantly from the initial seed in grain yield in the general direction of the mean of the contiguous populations. This suggests that when grown in proximity to each other a high-yielding line would tend to degenerate in yield, and a low-yielding line to increase. The once-grown seed used in the present test was obtained from the small rows of the previous year, and demonstrates the futility of using seed for experimental purposes which has not been isolated from other stocks. This equilibrium of populations growing together must result from



TEXT-FIG. 7.—Regression of once-grown seed on original seed in field bean.

cross-pollination, the effect of which would become apparent on introducing a new high-yielding line into a locality where yields are low, as in course of time the yield of the new line would fall to the general mean of the surrounding populations. This process was actually recorded nearly twenty-five years ago by Dr Hunter (Garner, 1945) who attributed it to cross-pollination between the new line and the existing populations.

Pod number does not appreciably alter under such conditions (table 5), and the number of seeds per pod does not show a significant difference when comparing original and once-grown seed. The only primary yield characteristic which appeared to be in any way highly heritable is seed size, and this was the only significant stock/seed source interaction noted.

5. DISCUSSION

The cytological irregularities observed at meiosis in this crop are those previously associated with inbreeding in naturally outbreeding crops such as rye, and have been reported upon. The distribution and occurrence of these irregularities in *Secale cereale* have been discussed by Müntzing and Prakken (1941) and Akdik and Müntzing (1949) in population material, and by Lamm (1936), Müntzing and Akdik (1948) in the first generation of inbred rye. Rees (1955) has shown that these irregularities during meiosis are directly attributable to the effect of inbreeding the normally outbreeding rye plant. Similar irregularities have been described for inbred *Alopecurus myosuroides* (Johnsson, 1944). Bridges and fragments, spiralisation defects, and a general timing unbalance have been observed in *Lathyrus odoratus* material that had been inbred for a number of generations (Upcott, 1937).

The similarity between these observations described for *Vicia faba* and those recorded on other plants is striking. A normal inbreeding plant would not be expected to suffer any cytological ill-effects following inbreeding so that it may be concluded that *V. faba* is in fact an outbreeding species. Its relatively high chiasma frequency, which appears to confirm this, would in fact be disadvantageous in a normal inbred plant where fitness, rather than flexibility, is to be desired (Darlington and Mather, 1949).

Further evidence for the basically outbreeding nature of *V. faba* is provided by the studies on the floral mechanism. Although the pollen is shed before the flowers open, this is effectively neutralised by the necessity for tripping before the pollen is able to germinate. In nature, tripping is carried out by insects, and invariably involves the deposition of foreign pollen on the stigmas. Due to the faster growth of foreign pollen, cross-fertilisation would almost always result from natural tripping. Taken in conjunction with this differential pollen growth, the self- and cross-sterility which appear to exist in varying degrees suggest an incompatibility system of an incomplete nature. Such a system has been demonstrated in lucerne (Whitehead and Davis, 1954), but cannot be explained on the basis of major gene effects alone.

It can be seen from the analysis of the experimental data that a number of factors have an important bearing on yield as a whole. Yield expression in field beans can be separated into three main phases of development, firstly, the period in the development of the plant up to and including flower formation ; secondly, the period from pod-setting until maturation of the crop ; thirdly, the phase of pollination and fertilisation which link together the other two periods and which is of paramount importance for seed production.

The data obtained for the vegetative characters of the plant show that in general they are positively correlated with each other. This

suggests that a strong and vigorous plant should also bear a large number of mature flowers, a feature borne out in the general observations. Pod formation, however, appears to be an independent process, and the crucial period must occur during pollination and fertilisation.

Although 60-70 per cent. of the seeds produced are the result of self-fertilisation, the genetic system is adjusted to a high hybridity optimum and would therefore require a high rate of outcrossing for its efficient working. It can be seen, table 2, that in the field only 8-9 per cent. of the total flowers formed appear to have been tripped by insects, since only 30-40 per cent. of those producing pods do so as a result of crossing. Either there is a shortage of tripping insects, due to adverse weather conditions, or the insects which are present are unable successfully to trip the flowers. In Great Britain only the larger species of bumble bees (*Bombus* spp.) are capable of tripping bean flowers with any degree of efficiency, and even then there is a tendency for certain bees to pierce the base of the corolla and remove the nectar without tripping the flower. The difficulty in tripping bean flowers may have resulted from the unconscious selection through husbandry practice resulting in greater flower size which as a result became less well adapted for the pollinating insects to work. This difficulty also appears in lucerne and other papillionaceous flowers, and may be one of the factors influencing the change over to self-fertilisation that has already occurred in *Pisum* and *Phaseolus*.

For the successful production of seed in beans the following four factors have to be considered :

(1) *The inherent fertility of the plant.* The basic capacity of any particular plant to produce seed is undoubtedly under polygenic control.

(2) *The tripping mechanism.* Until tripping has occurred pollen is unable to germinate, consequently insect visitation has to be chiefly relied upon for appreciable pod formation.

(3) *Differential pollen-tube growth.* Foreign pollen appears to germinate and grow faster than self pollen, although in the absence of foreign pollen self pollen must sometimes grow down to fertilise the ovule.

(4) *Incompatibility.* In all probability both self- and cross-incompatibility operate to some degree, although possibly under polygenic control, but the participation of major genes in their control cannot be ruled out, Lundquist (1954, 1955).

Under natural conditions these factors are subject to many environmental influences, and at the same time quite probably interact with each other. Basic fertility, like any other quantitative character, can be influenced by manurial treatment and improved by selection and breeding. Nectar production has been shown in alfalfa to influence the frequency of insect visitation (Pedersen, 1953), and therefore the degree and efficiency of tripping. The ideal, however, would be to

eliminate dependence on insects by the selection of a self-tripping, highly self-fertile plant.

In a crop cultivated for its grain, the breeding system should allow the production of a relatively homogeneous product. An outbreeding system is less of a handicap when dealing with forage crops, but field beans occupy an intermediate position. Two alternatives are open to the breeder, either to persevere with selection methods suitable for outbreeding plants, with the attendant difficulties of isolation requirements, or attempt to change the breeding system towards that of a true inbreeding plant so that cereal breeding methods can be used. At present, in *Vicia faba* inbreeding is imposed on an outbreeding system, but it should be possible to complete the change over to inbreeding.

Inbreeding experiments with field beans (Griffiths and Rowlands, unpublished data) suggest that although the general vigour of a population may deteriorate following continued selfing, certain individual plants appear none the worse and set seed quite well in the absence of pollinating insects.

Enormous simplification of the breeding work in the bean crop would result from the development of a self-tripping, self-fertile variety as a basis for subsequent strain building since such material would self-pollinate in preference to cross-pollinating and could therefore be expected to set seed in the field without difficulty. The success of such a programme would not become immediately apparent and in the meantime improvement will have to proceed in the direction of winter-hardiness and a reduction of pod-shattering.

6. SUMMARY

1. A cytological examination of natural populations of field beans revealed the widespread occurrence of irregularities during meiosis of a type previously associated with inbreeding depression in an outbreeding species.

2. The floral mechanism is adapted for cross-pollination and the differential pollen germination and growth in association with a weak incompatibility system suggests the limitation of self-fertilisation.

3. Under field conditions a major proportion of the seed produced results from self-fertilisation, the inadequacy of insect pollination forcing the plants to self with the consequent poor yield. It is therefore assumed that inbreeding is superimposed on a naturally outbreeding system.

4. Despite the high degree of self-fertilisation normally encountered in the field, the effect of cross-pollination in contaminating breeding material by other pollen is considerable, thus increasing the difficulties inherent in any bean breeding programme.

5. Enormous simplification of breeding work in the bean crop would result from the development of a self-tripping, self-fertile line as a basis for subsequent strain building.

Plate I

FIG. 1.—Normal metaphase of meiosis showing differences between *M* and *m* chromosomes.

FIG. 2.—Dividing nuclei showing stickiness due to nucleic acid.

FIG. 3.—Diakinesis showing fragment.

FIG. 4.—Early metaphase showing stickiness and extra chromosomes.

FIG. 5.—Stickiness and micronuclei during division.

FIG. 6.—Extra chromosome from breakage of the *M* chromosome.

FIG. 7.—Precocious separation of daughter centromeres.

FIG. 8.—Early anaphase with heterochromatic fragment.

FIG. 9.—Separation of chromatids at first anaphase and uncoiling of the chromosome thread.

All figures × *ca.* 2000.

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Plate II

FIG. 1.—Uncoiling of the chromosome thread and precocious separation of the chromatids.

FIG. 2.—Second anaphase immediately following the first, with uncoiling and separation of the chromatids.

FIG. 3.—Second anaphase with both *M* chromosomes involved in bridge formation.

FIG. 4.—First anaphase with bridges and heterochromatic fragments.

FIG. 5.—Micronuclei at second division.

FIG. 6.—Micronuclei at tetrad stage.

FIG. 7.—Second division bridge and micronuclei.

All figures $\times$ *ca.* 2000.

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GENOTYPIC CONTROL OF CHROMOSOME BEHAVIOUR IN RYE

VI. SELECTION FOR DISJUNCTION FREQUENCY

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1. INTRODUCTION

It has been shown (Thompson, 1956) that the frequency of disjunctional separation of the chromosomes in rings and chains formed at meiosis in an interchange heterozygote is subject to the control of the genotype. One would expect, therefore, that the disjunction frequency could be changed by selection, and indeed if the differences found between characteristic disjunction frequencies of closely related species (*e.g.* in *Œnothera*, Cleland, 1926 ; Kulkarni, 1929) are genetic, they suggest that selection may in fact bring about such a change. The following account offers direct evidence of the effect on this character of selection among genotypes within a species.

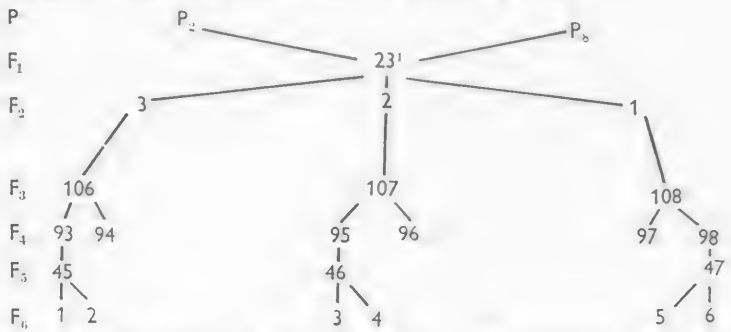

FIG. 1.—*Pedigree of material.* P_2 and P_8 are inbred lines. The parents and each number in F_1 and F_2 represent a single plant. In F_3 and subsequent generations each number represents a family which arose from a single plant self-pollinated in the preceding generation.

2. MATERIAL AND METHOD

The material used is that described by Thompson (*l.c.*). Two independent interchanges are involved, and these can be distinguished at diplotene in heterozygotes, though not at metaphase. Interchange A includes the nucleolar chromosome, and interchange B contains slightly unequal chromosomes.

The interchanges were found in an F_1 plant from a cross between two inbred lines of rye. This doubly heterozygous F_1 plant was selfed ; and among the F_2 plants three double heterozygotes were used to start three lines, each maintained by self-pollinating one double heterozygote in every generation. The pedigree is shown in fig. 1.

3. SELECTION

During the breeding programme, from F_3 onwards, the aim was to grow families of twenty-five plants, each family being obtained by selfing, where available, three heads on a single plant. Not all plants gave this number of seed, and thus there was selection for high seed set between plants within a family. In so far as the variation in self-fertility was due to differences in disjunction frequency, and was heritable, we might expect an increase in the frequency of disjunctional associations in subsequent generations. No selection was practised in the F_2 because the single plants in each line were chosen without reference to their seed set.

TABLE 1

The maximum likelihood estimates of mean disjunction frequency for interchange A (p_a) and interchange B (p_b) in the three lines and the number of plants involved in these estimates. The pooled estimate is obtained from data summed over the three lines. The three F_2 plants used to start the lines were not scored separately from other F_2 plants

	line (1)			line (2)			line (3)			Pooled estimate		
	p_a	p_b	No. of plants	p_a	p_b	No. of plants	p_a	p_b	No. of plants	p_a	p_b	No. of plants
F_1	...	...	...	...	...	...	...	...	...	0·88	0·88	2
F_2	...	...	...	...	...	...	...	...	...	0·66	0·55	11
F_3	0·56	0·47	7	0·75	0·67	17	0·74	0·82	14	0·71	0·69	38
F_4	0·67	0·76	26	0·82	0·79	26	0·80	0·87	14	0·77	0·79	66
F_5	0·69	0·63	11	0·84	0·81	9	0·75	0·75	5	0·78	0·71	25
F_6	0·67	0·78	9	0·86	0·79	27	0·81	0·78	29	0·80	0·79	65

4. RESULTS AND ANALYSES

As a result of segregation in the double heterozygote, selfed to produce succeeding generations, each family may be classified into four types with respect to the interchanges.

(i) Plants heterozygous for both interchanges (Class AB).

(ii) Plants heterozygous for A interchange, homozygous for B (Class A).

(iii) Plants heterozygous for B interchange, homozygous for A (Class B).

(iv) Plants homozygous for both interchanges (Class O).

The mean disjunction frequency for interchange A may then be obtained by pooling data from all plants in class A. A similar procedure will give the mean frequency of disjunction for interchange B. However, it will be seen that information about these values is also contained in the double heterozygotes (Class AB), but since the two interchanges are indistinguishable at metaphase this information is not readily extracted. Efficient estimates, taking into account observations from the three classes (A, B, and AB), may be obtained by the method of maximum likelihood (Thompson, *l.c.*). In all, two hundred

and seven plants are involved. These estimates, and the joint estimates over the three lines in each generation, are given in table 1.

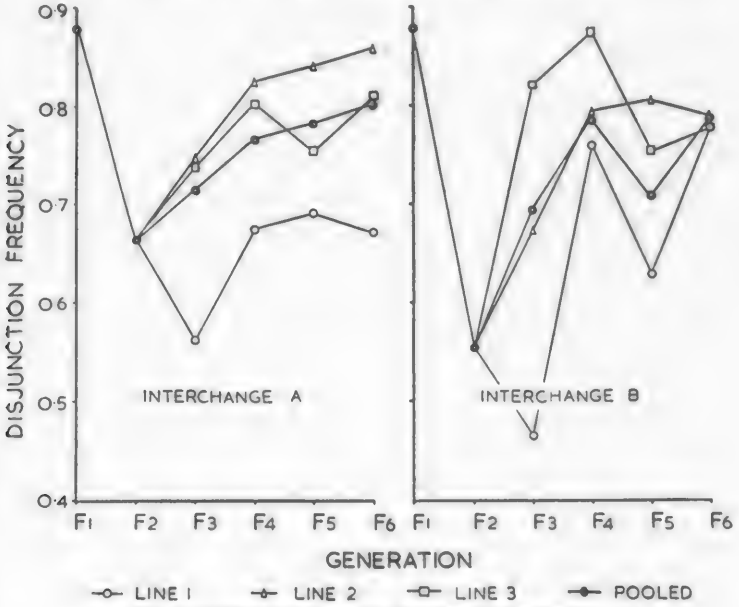

FIG. 2.—Disjunction frequency plotted against generation.

These estimates have also been plotted against generation in fig. 2. In the graph it will be seen that from F_3 onwards the disjunction frequency in general increased for both A and B interchanges. We

TABLE 2

Regression analysis of variance. (*Disjunction frequency on generation*)

	Item	N	M.S.	V.R.	P
(i)	Mean regression	1	0·02627	6·4	0·05-0·01
(ii)	Heterogeneity in regressions of lines	2	0·00982	2·4	0·2-0·1
(iii)	Heterogeneity in regressions of interchanges	1	0·00002	...	...
(iv)	Heterogeneity in means of lines	2	0·05034	12·2	<0·001
(v)	Heterogeneity in means of interchanges	1	0·00008	...	...
	Error	16	0·00412	...	...
	Total	23	...	...	...

must, however, analyse the data in more detail and test the significance of this increase. This has been done by means of an analysis of variance based on the regression of disjunction frequency on generation. The result of this analysis is shown in table 2. Since no selection

was practised on the F_2, only data from generations subsequent to this are analysed.

Item (i) in table 2 shows that there is a significant joint regression over the three lines and for both interchanges. ($P = 0{\cdot}05$-$0{\cdot}01$). Furthermore, as the hypothesis demands, this regression is positive ($b = +0{\cdot}0296$). There can be little doubt therefore, that the disjunction frequency has increased from F_3 to F_6. From this we can conclude that selection for high seed set resulted indirectly in an increase in disjunction.

Two alternative explanations for these trends must be considered. Firstly, the change could be due to seasonal influence. However, it is extremely unlikely that the seasons 1953 to 1956 were so progressively and increasingly favourable to higher disjunction. Secondly, the results could be explained as an accessory of inbreeding depression. This can be ruled out by comparing the F_1 and F_2 values. The F_1 had a higher disjunction frequency than any generation in all lines. The F_2 had a low disjunction frequency, generally lower than other generations.

It will be seen that though there is no evidence in these data of differences in the rate of response to selection between the three lines, they show marked differences in their mean disjunction frequencies (table 2, items (ii) and (iv)), whereas the two interchanges apparently not only behave similarly, but have the same mean values (table 2, items (iii) and (v)) The striking differences in the means of lines support Thompson's earlier findings in F_3 and F_4, which he attributes to segregation for genes controlling the character, and show moreover that the divergence in lines has been maintained. In these data the consistently uniform behaviour of the two interchanges in all lines suggest that they are controlled by the same genic system and respond similarly to it (*cf.* Thompson *l.c.*).

5. CONCLUSIONS AND SUMMARY

During an inbreeding programme the disjunction frequency of interchange heterozygotes increased by about 10 per cent. from F_3 to F_6. This increase resulted indirectly from selection within families for high self-fertility. No doubt in natural populations which contain interchanges either floating as in *Campanula* (Darlington and Gairdner, 1937) or fixed, as in *Œnothera* and *Rhoeo*, such selection has been of great importance in their survival. It may equally well explain the very high disjunction frequencies found recently in populations of cockroaches (Lewis and John, 1957).

A marked difference between lines in the material supports the earlier findings of Thompson. No differences between interchanges was observed, suggesting that they are controlled by the same genic system and respond similarly to it.

Acknowledgments.—I am much indebted to Prof. K. Mather, F.R.S., and Dr H. Rees for continual advice and criticism, and to Mr J. B. Thompson for the use of his data.

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COMMENT AND REVIEWS

COMMENTS ON D. V. LINDLEY'S REVIEW OF SIR RONALD A. FISHER'S "STATISTICAL METHODS AND SCIENTIFIC INFERENCE"

Statistical Methods for Research Workers was first published in 1925. In 1950, to mark the twenty-fifth anniversary of its publication, I was asked by the editors of the *Journal of the American Statistical Association* to make an appreciation of the influence of the book on statistical methods. In the course of preparing this article (Yates, 1951) I took the opportunity of reading the reviews of the first edition. Lindley's review (1957) of Fisher's new work, *Statistical Methods and Scientific Inference*, shows a reaction which is very similar to that shown in some of these reviews. But, as Lindley himself admits, this and the further book *The Design of Experiments* "laid the foundations of statistical science as we know it to-day". Had Lindley studied these reactions and the background that gave rise to them he might have been hesitant in committing to print his concluding remarks :

". . . the best that we can hope for is that the book will make statisticians realise the unsatisfactory nature of the Fisherian argument and make them more ready to read and accept the Bayesian argument. The worst that can happen is that it should command an important position just because of brilliant work done by the author thirty years ago."

Why has Lindley found so little of value in this new work ? I think it is because he himself, like so many mathematicians trained in the formalities of theoretical statistics and not working in direct contact with scientific research workers, has no direct experience of the type of inductive inference that is in fact required in the course of scientific research. Thus at the outset of his review he states :

". . . it is clear that statisticians to-day are much happier in designing or analysing experiments than they are in explaining just why they are doing what they do. For example, new significance tests are continually being produced but the concept of a significance level is not clearly understood."

This, I would submit (as one who has spent a considerable amount of time designing and analysing experiments in association with the scientists concerned) gives a completely false picture of the real situation. Statisticians who have experience of experimental design and analysis are very clear why they do what they do, and if they have a gift for exposition can explain it when necessary. It is the mathematical statisticians without such experience who spend their time producing new significance tests which have no relevance to the material under examination. Thus, for example, M. G. Kendall in his *Advanced Theory of Statistics* (1943) takes as an example the data of a simple factorial experiment (a $2 \times 2 \times 2$ design with confounding of the 3-factor interaction) given by me specifically to illustrate the design and analysis of experiments of this type (Yates, 1935), re-analyses it using a number of procedures that any expert in the field would regard as incorrect, and in consequence makes what is in effect a different test of

significance, the results of which in fact disagree with my own. Yet he nowhere considers it worth while to inform his readers of this discrepancy in method and conclusions !

It is, I think, this failure to appreciate the type of inductive reasoning that scientists follow and criteria that any system of inductive inference must satisfy if it is to be of any real use, that has resulted in a complete failure by Lindley to recognise the new contributions that the present work has made to the subject.

It is not my purpose in this note to undertake a review of the book nor to enter into detailed discussion of the issues raised by Lindley in his review, many of which are better discussed in journals devoted to mathematical statistics. There are, however, a few points on which I would like to comment.

From the passage quoted above it is apparent that Lindley favours the Bayesian argument. Yet he nowhere comments on the objections to it that have been made for many years by Fisher, and which are summarised and amplified in the present work. Perhaps his idea that he has convicted Fisher of a " mathematical error " has led him to conclude that no further refutation of the concept of fiducial probability is necessary and that as the Bayesian solution is the only alternative that is available this must forthwith be accepted. This is a poor argument for the Bayesian solution !

As for the " mathematical error " itself, this accusation appears to be based on a disregard of the principles Fisher lays down in his book. It is of course well known that if, for example, the full data from a sample from a normal distribution is available this cannot be efficiently combined with the data from a second sample merely by using the value of t in the first sample. But in such a case the values of $\bar{x}$ and s^2 serve to demarcate recognisably different sub-sets. If the value of t is all that is known concerning the first sample—as might indeed be the case if the data were reported by another worker—then the corresponding fiducial probability could be correctly used as a prior probability.

Lindley complains that Fisher has made " an ingenious attempt " to present an argument for significance tests which avoids consideration of alternative hypotheses by taking an example in which the probabilities of more extreme events are negligible. Actually in this example no real problem arises since only a single tail is involved. The subsequent treatment of composite hypotheses and the use of likelihood instead of significance for discrete observations surely removes the need for detailed discussion of what observations are to be considered more extreme in such cases.

Lindley also objects to the fact that geneticists take no account of the number of chromosomes when making a test of significance for linkage. This would seem to me to be likely to lead to just the sort of confusion of evidence that usually results from the introducton of prior probabilities. Chromosomes are of very different length, their number is not always exactly known, and there is no reason to expect that the genes entering into linkage studies are a random selection of all genes. The question asked by the geneticist is therefore rightly : are the values of the observed frequencies such as might arise if the linkage were zero ? The assessment of the evidence on the assignment of genes to the different chromosomes comes later.

Finally, Lindley criticises Fisher for not making adequate reference to

contemporary writers and for apparent ignorance of their work. I do not know whether Fisher has in fact studied all the references (including one unpublished thesis !) that Lindley cites—I certainly have not—but surely a word of thanks is rather due to him for the extremely interesting historical review of the development of ideas on probability. F. YATES.

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REVIEWS

THE ORIGIN OF LIFE ON THE EARTH. By A. I. Oparin, translated from the Russian by Ann Synge. Third and Revised and Enlarged Edition. Oliver and Boyd, London. 1957. Pp. 495. 35s.

This is the third and greatly enlarged edition of the famous Russian book which was first published in 1938. It appears at a time when the Russians have been highly successful in their first stages of determining whether there is life on other planets and it may well be that the next edition will need to have an extended title !

Many readers, particularly the less specialised ones, will find this a popular and quite fascinating book on a tantalising subject. In the earlier chapters the author reviews, in an exhaustive manner, the history of the many efforts by which the philosophers and early investigators sought either by the theoretical approach or by experimentation to solve the riddle of the origin of life. The treatment of such matters as spontaneous generation is not highly critical but it makes fascinating reading. One wonders, however, whether the description of the work concerning attempts to construct models of living organisms is worth perpetuating despite the pretty pictures of artificial fungi and algæ.

The summary of evolutionary theories on the origin of life is concise and excellent. In Chapter IV there is provided a long and altogether feasible account of the possible mode of formation on earth of simple organic substances. The author marshals the ideas taken from a diverse range of papers, particularly from authors in the Soviet Union, and from Urey's writings, showing that hydrocarbons could be made abiogenically. He gives also an interesting chapter providing evidence for the presence of hydrocarbons in various planets as determined by spectrographic methods. These chapters are again not highly critical but it is clear that the author holds firmly to the theory that simple organic substances had a chemical origin and he amplifies and extends his views in a plausible and logical way in a further discussion of oxidising and reducing conditions of the lithosphere.

The possible sources of the great amount of energy needed for the chemical synthesis of carbon compounds are discussed at length and the account makes fascinating reading. Ultra-violet light played its full part in these chemical reactions and Oparin deduces that the formation of more complicated organic compounds occurred mainly in the waters, in the seas and in the oceans. He is well aware that the substances catalysing the reactions must have been salts in aqueous solution or in the insoluble deposits. In view of recent developments in polymer formation at low temperatures by inorganic reagents, surface catalysis merits closer attention by future research workers.

The author approaches the important question concerning the mode of synthesis of dissymmetric molecules in a cautious manner and would appear to favour among other things the possibility of using dissymmetric crystals as catalysts. He evidently likes the outmoded idea of making sugars from formaldehyde and states categorically that sugars arose primarily on the waters of the earth's surface long before the appearance of life on it.

Ideas on the formation and structure of other biologically important compounds, particularly those containing nitrogen, receive a good deal of

attention. The question of amino acids both from the viewpoint of their structure and their origin comes in for careful review. Indeed the chapters on the origin of structures and functions in regard to physiologically active compounds, particularly proteins and nucleic acids, are amongst the most interesting and best described in the book and they will be most useful to the non-specialist reader. Clear accounts are given of ideas on the development of organic multimolecular systems and the author makes good use of the idea of coacervates or colloidal macromolecules as being ideal initial systems in which self duplication of molecules could begin.

Theories on the origin of enzymes and on the first organisms are set forth in an attractive manner and there is no doubt that the literature has been surveyed in a thoroughly exhaustive way.

Further chapters on energy, metabolism, photochemical reactions, photosynthesis and on respiration bring the subject matter up-to-date from the biochemical point of view. It is important to note that all chapters include many important references to Russian work which has hitherto been inaccessible to most of the world.

To those of us who have had the good fortune to meet Professor Oparin in his own laboratory and to talk with his enthusiastic young colleagues, this book is particularly welcome for his genial personality pervades every page. He has assembled the published claims and his own life's work in a masterly way and has provided thereby much food for thought and stimulation to young workers who are interested in this very important biological field. The book, which is cleverly and sympathetically translated by Ann Synge, must find an important place in every science and general library. It is beautifully printed and arranged and the photographs reproduced are of considerable interest.

However, some specialists will not find the book sufficiently critical and may not like some of the " paper chemistry ". On the first reading some non-specialists may find the description of the complexity of living things and their origin somewhat bewildering. Moreover, since this is a singularly God-less book some readers may need to regain equilibrium by reading in all its beautiful simplicity the First Chapter of Genesis ! M. Stacey.

CONFERENCE ON CHROMOSOMES. Lectures held at the Conference on Chromosomes, Wageningen, 16th-19th April 1956. Willink, Netherlands. Pp. 231. f. 8.10.

This book contains the papers presented at the first of a series of triennial conferences organised by the Agricultural University and intended to discuss fundamental subjects which would be of interest to every scientist. It contains seven papers :—" Die Chromosomen struktur in kern während der kerntielung und der entwicklung des organismus " by E. Heitz ; " Some aspects of the chemical structure of proteins and nucleic acids " by H. D. Springall ; " Cytochemistry of nuclear elements " by T. Caspersson ; " The recognition, distribution and action of nucleic acids " by N. W. Pirie ; " Studies on the mutation process in plants—regularities and intentional control " by L. Ehrenberg, A. Gustaffson and D. von Wettstein ; " Chromosomes in relation to species differentiation and plant breeding " by A. Müntzing ; and " Messages and movements in the cell " by C. D. Darlington.

As is inevitable in the nature of the subject and evident from the titles of the papers, the conference proceedings do not in any real sense make a book but rather a heterogeneous collection of papers. Those by Ehrenberg *et al.* and by Müntzing, stand apart, both being primarily concerned with aspects applicable to plant breeding. They are interesting accounts of work of these authors but bear little or no relation to the other papers.

The papers by Springall, Caspersson and Pirie concern the structure, location and interrelations of nucleic acids and proteins in the cell. That by Heitz concerns chromosome variation between species and (Balbiani-rings) in differentiation, and that by Darlington is a broadly synthetic discussion of the inferences that can be made concerning modes of integration of cell activity.

The papers by Springall, Pirie and Caspersson, and references in Darlington's paper to matters concerning nucleic acids might have made an integrated series. In fact, however, perhaps because the discussions that must have taken place are not included in the volume, these papers fail to connect except in a negative sense. Springall's beautifully clear factual account of the evidence on which our present knowledge of nucleic acids and protein structure is based, is a model contribution. Pirie follows with a hypercritical paper from which we might conclude that none of the information we have concerning the distribution or functional relationships of nucleic acids and proteins in the cell has any sure basis, but from Caspersson's paper we would conclude that a great deal is established concerning these matters. This conflict makes it less easy to follow Darlington's stimulating contribution, at least whenever it touches on matters concerning nucleic acids.

The trouble arises from Caspersson's and Pirie's papers. Caspersson makes no attempt to meet any of the criticisms of his techniques such as those Pirie expresses, nor to discuss the problems that arise in the reader's mind when Caspersson's results seem in disagreement with those found by other authors. Pirie produces all the evidence he can to show that our knowledge stands on shaky foundations but makes no attempt to assess the relative weight we might put on them. For example, does Pirie really think that the demonstration that " the chromosomes in tissue sections exposed to the coloured products made by carrying out the Feulgen reaction in a test tube stain characteristically " has any relevance, since Howard and Pelc (whom Pirie does not quote) demonstrated that DNA-contained labelled phosphorus is to be found predominantly in the chromosomes? Surely the only relevant evidence that remains against the view that unfixed chromosomes contain large quantities of DNA is that of Chayen and Norris, who claimed that most of the " chromosomal " DNA moved from cytoplasm to nucleus " during the operation of normal histology ". Pirie quotes but does not assess this claim, and nobody else refers to it. If it is sound, we are lost in the paper of Caspersson and parts of Darlington's, and all that has been written about nucleic-acid function needs review. An attempt in this book to discuss where the evidence stands would have made it a book. As it is we are presented with seven papers, each of value, but none helping us with the others. Their inclusion together in a single volume without discussion has only one value: it points to the need for a broad constructive discussion of the validity of the evidence available.

J. M. Thoday.

THE DARWIN READER. Ed. by Marston Bates and P. S. Humphrey. Macmillan, London. 1957. Pp. 481. 30s.

An anthology of Darwin's writings combined with a valuable bibliography and notes. Its special characteristics are that it abridges the enlarged sixth edition of the *Origin of Species* (1872). It includes the 1858 correspondence but it omits any extracts from the four books which include his experimental and forward-looking work on plants. Perhaps intentionally it omits reference to Darwin's outstanding mistakes. And it omits his clearest statements on human heredity (in *Animals and Plants*) possibly because the editors think they are mistakes. It includes only one of his thousand or two published letters and it omits reference to Keith's *Darwin Re-valued* (London, 1954) and the anonymous *Autobiography* (New York, 1950). There is a good index which however refers the reader to evolution where Darwin did not use the word (in the *Origin*) and not where he did use it (in the *Descent of Man*).

For all its shortcomings we must be grateful to the editors and publishers for a well-produced and much needed book. C. D. D.

SHORT REVIEWS

EFFECT OF RADIATION ON HUMAN HEREDITY : Report of a Study Group convened by the World Health Organisation together with Papers presented by Various Members of the Group. Geneva. 1957. Pp. 168. £1.

An account by twelve authorities of the data that will have to be considered and the methods that will have to be used in attempting to discover the genetic effects of irradiation on human populations and to determine whether these effects are advantageous or disadvantageous to the populations that are exposed to radiation.

THE HUMAN SUM. Ed. by C. H. Rolph. Heinemann, London. 1957. Pp. 232. 18s.

An account of population control and family planning chiefly in England, by ten authors, delightfully decorated by ironical cartoons. Genetic aspects are omitted. There is an appendix attempting to reconcile social needs with the doctrine of the Church of England and *vice versa*.

BLOOD ROYAL. I. Moncreiffe and D. Pottinger. Nelson, London. 1956. Pp. 64. 12s. 6d.

Under the disguise of a brilliantly illustrated children's book of kings this learned work on the European royal families explains their racial origins and erratic breeding system with its unexampled alternation of inbreeding and outbreeding and of course its unexampled documentation.

MAN'S JOURNEY THROUGH TIME. L. S. Palmer. Hutchinson, London. 1957. Pp. 184. 30s.

An attempt to link the development of man recorded by fossil remains of bones with that recorded by history and pre-history. The attempt, which is hazardous in any case, is made more so by the author's neglect of the vast evidence of present variation.

ADDENDUM : *Theoretical Genetics* by R. B. Goldschmidt, reviewed in *Heredity*, *10*, p. 434, is issued in Britain by the Cambridge University Press.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND TWENTY-FIFTH MEETING of the Society, held on 23rd NOVEMBER 1957, at UNIVERSITY COLLEGE, LONDON

CHROMOSOME POLYMORPHISM IN THE COMMON SHREW

C. E. FORD and J. L. HAMERTON
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The chromosome numbers of approximately 100 common shrews trapped at seven localities in the British Isles were found not to be constant, but to vary between individuals from 21 to 27 in males and from 20 to 25 in females. The situation is complicated by the presence of a multiple sex chromosome system. Males are $X\,Y_1\,Y_2$; females are XX. The autosomes comprise six constant pairs of metacentric chromosomes, and three pairs of elements each of which may occur either as one metacentric or as two acrocentric chromosomes. Variation of chromosome number is therefore of the "fragmentation-fusion" or "Robertsonian" type well-known in several species of grasshoppers and beetles. The variable autosomes are the sixth, seventh and eighth in order of decreasing length. The metacentric element 6 is easily distinguished; metacentric elements 7 and 8 are both short, with median centromeres, but are distinguishable from one another in good preparations. Of the $3^3 = 27$ possible cytological types (eight of which are triple homozygotes) 19 have been identified. Evidence gathered during the past summer indicates: (1) A marked change from the previous year has occurred in the composition of the principal population studied. (This might have been influenced by heavy trapping.) (2) Sharp differences may exist between populations living within relatively short distances of each other. (3) Many, and perhaps most, British populations are triply homozygous for one or other of the eight possible combinations.

THE ACTION OF TRIETHYLENE MELAMINE (TEM) ON THE FERTILITY OF MALE MICE

B. M. CATTANACH and R. G. EDWARDS
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Large doses of TEM induce sterility almost immediately in male mice. Sperm, however, is present until 38 days after treatment. Few if any sperm can then be seen for a further 30 days. Smaller doses result in sterility within three days and this is maintained for at least two weeks. Daily examination for the number of embryos which implant in females mated during this period shows a characteristic curve. This is thought to be the result of differences in sensitivity of the various stages of spermatogenesis. Thirty per cent. of the offspring produced from the early fertile matings carry translocations.

MAINTENANCE OF SEGREGATION OF BLOOD GROUP GENES DURING INBREEDING IN CHICKENS

D. G. GILMOUR
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Six independent loci determining antigens of the red blood cells were segregating in the fourteenth successive brother-sister matings of the RE line of White Leghorn

chickens. Four of these correspond with the *A*, *B*, *C*, and *D* loci of American workers, the other two (*L*, *N*) are new. Although further loci might be fixed in isozygous condition in the RE lines and three other lines studied, attempts to identify them by making immunisations both between backcross progeny and between lines were unsuccessful.

In the eighteenth generation only three loci were still segregating. Fixation of the other three loci occurred over a period when only two males were used to reproduce the line in each generation, instead of five as formerly.

Direct evidence indicates that for each of four loci considered separately, heterozygous individuals were superior to homozygotes in reproductive ability, and hence were at an advantage in a system of selection in which the largest groups of sibs were chosen to continue the line. Heterozygous sires were superior in fertilisation rate, heterozygous dams in egg production, and heterozygous progeny in viability. The magnitude of these effects varied between loci, but *L* was the most powerful. Comparisons involving the *N* locus have not yet been possible, although the fact that it is still segregating would imply that it has similar effects. The *D* locus became fixed early in the study.

THE DISTRIBUTION OF HÆMOGLOBIN AND BLOOD POTASSIUM TYPES IN BRITISH BREEDS OF SHEEP

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and
H. HARRIS and F. L. WARREN
London Hospital Medical College, London

Sheep have been found to be polymorphic for two genetically determined characters: type of adult hæmoglobin, and concentration of potassium in the red blood cells. The distribution of these characters has been studied in samples from 33 British breeds. While there is no association between the two characters within breeds, there is a remarkable correlation between breeds, a high frequency of the hæmoglobin A gene being associated with a high frequency of the HK gene.

The possible causes of the observed pattern of gene frequency distribution are discussed.

THE PERFORMANCE OF SHEEP WITH DIFFERING HÆMOGLOBIN AND POTASSIUM BLOOD TYPES

J. W. B. KING
Animal Breeding Research Organization, Edinburgh, 9
J. V. EVANS
Rowett Research Institute, Bucksburn, Aberdeenshire
and
H. HARRIS and F. L. WARREN
London Hospital Medical College, London

Records of sheep with differing blood types have been examined in a large flock of Scottish Blackface sheep. Differences in reproductive performance, which would be subject to natural selection, and in growth rate, fleece characteristics, etc., which would be subject to artificial selection, were sought as an explanation for the gene frequencies observed in different breeds. No significant differences were found in any weights or measurements, nor in the number of lambs produced by different types of ewe.

PHENOTYPIC FLEXIBILITY IN PLANTS

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Phenotypic flexibility, *i.e.* the ability of a genotype to vary its expression in different environments, is a character of considerable adaptive significance in most plants. Cultivation of natural populations of the grass, *Agrostis tenuis*, shows that the adaptive changes in morphology caused by the environment are often twice or three times as great as those caused by the genotype. Phenotypic flexibility must therefore be considered to be as significant an attribute of plants as any other character.

A brief survey of related plant species shows that they may differ very greatly in their phenotypic flexibility. There is some evidence that the same is true of different populations within one species. Phenotypic flexibility must therefore be a character with an ordinary genetic basis. Its occurrence in species and in populations will be under the control of the normal processes of natural selection.

INDUCED MALE STERILITY IN *SILENE*

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In the long-day *Silene pendula*, partial or complete male sterility may be induced photoperiodically. Maximum male fertility is obtained when an interlude of forty or more short days is given early in life, and minimum when plants are exposed continuously to long days. In the extreme case of male sterility, the flower is effectively unisexual, with the stamens represented by minute papillæ. When some development of the androecium proceeds, the anthers may abort after the differentiation of the archesporium, or meiosis may be completed and the pollen fail to form subsequently—a failure generally heralded by early dissolution of the tapetum.

The partial or complete suppression of the androecium in long days is accompanied by a reduction of the corolla and a precocious development of the gynoecium with the early exsertion of the stigmata. The characteristics of the male-sterile flowers are thus directly comparable with those of "female" flowers of many genetically polyoecious flowering plants of different families in which unisexuality has originated directly from hermaphroditism. Evidently a common physiological basis may be involved, and the nature of the link is indicated by the fact that closely similar effects may be induced by the administration of synthetic auxins.

REVERSAL OF GENETIC DWARFISM IN PLANTS BY GIBBERELLIC ACID

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Gibberellic acid is a metabolic product of a plant pathogenic fungus, *Gibberella fujikuroi*. It has plant growth promoting properties; when applied to shoots of growing plants it usually accelerates internode extension and may increase leaf-growth, in both cases mainly by increased cell-extension. It has other physiological effects, including (1) breaking of dormancy of various plant organs; (2) induction of parthenocarpic fruit development; (3) induction of flowering of cold-requiring plants without vernalisation and of long-day plants in short days. A further effect, of greater interest to geneticists, is that it will induce normal growth of genetic dwarfs of several plant species (notably species of *Pisum*, *Lathyrus* and *Zea*). This observation has led to the hypothesis that plants must contain a hormone similar to gibberellic acid in its effects, and that some derangement in metabolism of this hormone is responsible for dwarfism. The presence of such a hormone has been

proved by actual extraction in several plant species, but the metabolic background of dwarfism is not yet understood. Genetical considerations also suggest that gibberellic acid may act by masking or reversing the effects of some growth-inhibitory system in plants and this view also is supported by physiological evidence.

GENETICS OF ANENCEPHALY

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Facts concerning the ætiology of anencephaly are presented. Marked geographical differences in frequency are observed. The incidence in some parts of the British Isles is 50 times that found in some parts of France. The uneven geographical distribution of cases must be taken into account when discussing possible causes, both environmental and genetical. Local peculiarities in water supply or in prevalence of infectious diseases could be of significance. It is concluded that recessive inheritance probably accounts for some cases. Others may be caused by chromosome translocation. Fresh mutation can occasionally be causal. Variations in susceptibility, dependent upon more than one gene, are not improbable.

HYPOPHOSPHATASIA, EXCRETION OF ETHANOLAMINE PHOSPHATE IN CLINICALLY NORMAL RELATIVES

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Hypophosphatasia is a rare osteodystrophic disorder. Biochemically it is characterised by a marked reduction in the level of serum alkaline phosphatase activity and a greatly increased excretion of ethanolamine phosphate in the urine.

The families of 14 individuals presenting with this condition have been investigated. No instance of parental consanguinity was observed. Out of 13 sibs of the propositi, two were diagnosed as having hypophosphatasia. No cases of the disease were found among other close relatives. Slight but definitely abnormal levels of ethanolamine phosphate excretion were observed in the urine of some members of the families. It is suggested that the clinically affected individuals are homozygous for a rare gene which in the heterozygotes leads only to a minor biochemical abnormality. If so, about 60 per cent. of the presumed heterozygotes can be detected by the chromatographic methods used.

The severity of the disease and the age at which the osteodystrophy becomes apparent are very variable. In our own, and in published material, sibs resemble each other rather closely in these respects. This suggests that more than one gene may be involved. Further heterogeneity in the material is revealed by the fact that the heterozygous manifestation rate is different in different sibships.

TASTE THRESHOLD FOR P.T.C. IN THYROID DISEASE

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Harris, Kalmus and Trotter, in a preliminary communication (*Lancet*, 1949), found a 40 per cent. incidence of non-tasters in adenomatous goitre. The frequency of non-tasters in a control series and also in diffuse goitre was found to be 30 per cent.

This work has been extended in a survey of patients (at present numbering about 400) attending the thyroid clinic at the David Lewis Northern Hospital, Liverpool. All the individuals investigated have had partial thyroidectomy carried out and pathological reports are therefore available in each case of the series.

Similar results have been obtained to those of Harris, Kalmus and Trotter in 1949.

The findings will be discussed.